Hormonal Regulation of ATP Binding Cassette Transporters

Dissertation zur Erlangung des Doktorgrades

der Naturwissenschaften (Dr.rer.nat.)

der Fakultät für Biologie und Vorklinische Medizin

der Universität Regensburg

vorgelegt von

Mohammed Ahmed A. Taher

Regensburg, im April 2004

Promotionsgesuch eingereicht am:

Die Arbeit wurde angeleitet von: Prof. Dr. med. Gerd Schmitz

Prüfungsausschuß: Prof. Dr. Rosemarie Baumann

Prof. Dr. med. Gerd Schmitz

Prof. Dr. Eggehard Holler

Prof. Dr. Stephan Schneuwly

Acknowledgements

In the name of ALLAH, the benificial, the merciful and most gracious. Before and after any thing, I am very grateful to ALLAH who gave me the effort and patience to produce this thesis.

This thesis was performed from August 2000 until February 2004 under the observation of Prof. Dr. med. Gerd Schmitz, Dr. Thomas Langmann and Prof. Dr. Eggehard Holler in the Institute of Clinical Chemistry at the University of Regensburg.

I am very grateful to Prof. Dr. med. Gerd Schmitz for his support in my work and for giving beneficial and essential advices and discussions.

My sincere thanks are to Prof. Dr. Eggehard Holler who offered help in reviewing this thesis.

I gratefully acknowledge the support of Dr. Thomas Langmann. He was my teacher in molecular biology and largely contributed to the completion of this thesis.

I want to express my thanks to all my colleagues especially to Dr. med. Wolfgang Drobnik, Dr. Alfred Böttcher, Dr. med. Ashraf Dada, and Dr. Gerhard Liebisch for their support and advices and also Manfred Haas, Wolfgang Hauer and Jolante Aiwanger for technical assistance.

Abbreviations

18srRNA	18S-ribosomal RNA
a	Adenin
aa	Amino acid/s
ABC	ATP binding cassette
ACAT	Acyl-coenzyme A cholesterol acyltransferase
ACTH	Adrenocorticotrophic hormone
AD	Alzheimer disease
AP-1	Activating protein-1
Apo	Apolipoprotein
ATP	Adenosine triphosphate
AZ	Alzheimer disease
BAT	Brown adipose tissue
bp	Base pair
BSA	Bovine serum albumin
c	Cytosine
cAMP	Cyclic 3',5'-adenosine monophosphate
cDNA	Complementary Deoxyribonucleic acid
CETP	Cholesterol ester transfer protein
CFTR	Cystic fibrosis transmembrane conductance regulators
CHD	Coronary heart disease
Ci	Curie
CMOAT	Canalicular multispecific organic anion transport
CSF	Colony stimulating factor
CT	threshold cycle
CYP27	Cytochrome P27
DNA	Deoxyribonucleic acid
DBN	DNA binding domain
DHEA/S	Dehydroepiandrosterone /sulfate ester (DHEAS)
DM	Diabetes mellitus
DMEM	Dulbecco´s modified Eagle´s medium
DR-4	Direct repeat sequences seperated by 4 nucleotides
EDTA	Ethylendiamin –Tetraacetat
EIF2	Eucaryotic initiation factor 2
E-LDL	Enzymatic modified LDL
ER	Estrogen receptor
ERK	Extracellular regulated kinase
FHD	Familial HDL deficiency
FAS	Fatty acid snthetase
Fra2	Fos related antigen 2
g	Guanin
GnRH	Gonadotrophin- releasing hormone
GR	Glucocorticoid receptor

GS-H	Reduced glutathione
h	hour
HBD	Hormone binding domain
HDL	High density lipoprotein
HepG2	Human hepatoblastoma derived cell line
HL	Hepatic lipase
HMG-CoA	Hydroxymethylglutaryl coenzyme A
HRT	Hormone replacement therapy
Hsp	Heat shock proteins
ICAM	Intracellular adhesion molecule
IGF	Insulin-like growth factor
IL	Interleukin
INF-γ	Interferon-γ
IRS	Insulin receptor substrate
KAP 1	Kruppel-associated protein 1
kDA	Kilodalton
KIR	Potassium inward rectifiers
LDL	Low density lipoprotein
LH	Luteinizing hormone or lutrophin
LPS	lipopolysaccharide
LXR	Liver X receptor
Mac.	Macrophages
MAP	Mitogen activated protein
M-CSF	Macrophage CSF
MDR	Multidrug resistance
MEK	MAP extracellular related kinase
MHC	Major Histocompatability Complex
NBD	Nucleotide binding domain
NPY	Nucleus neuropeptide Y
NTCP	Na^+/taurocholate cotransporting peptide
OABP	Oligoadenyl binding protein
OB	Leptin (the product of OB gene)
OB-R	OB receptor
OCT 1	Organic cation transporter 1
PAF	Platelet activating factor
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PFIC	Progressive Familial Intrahepatic Cholestasis
PI(3)K	Phosphatidyl inositol 3-kinase
PPAR	Peroxisome proliferator-activated receptor
Raf	MAP kinase kinase
Rho-GTP	Rho-guanosine triphosphatases
RLP	Reminant like lipoprotein
RNA	Ribonucleic acid

RNase	Ribonuclease
rpm	Rotate per minutes
RT	Room temperature
RT-PCR	Reverse transcription-PCR
RXR	Retinoid X receptor
SCAN	**S**RE-ZBP, **C**T-fin-51, **A**W-1 and **N**umber 18 cDNA
SDP1	Scan domain containing protein 1
SDS	Sodium dodecyl sulfate
Ser	Serine
SMC	Smooth muscle cells
SR-B1	Scavenger receptor class B1
SREBP1c	Sterol Regulatory Element Binding Protein 1c
SRIF	Somatotropin releasing inhibiting factor (somatostatin)
SS	Somatostatin
t	Thymin
T3	Triiodothyronine
T4	Tetraiodothyronine
TAP	Tissue antigen presentation
Taq	Thermophilus aquaticus
TBS-T	Tris- buffer- saline with 0.1% tween
TD	Tangier disease
TMD	Transmembrane domain
TNF-α	Tumor necrosis factor - α
TR	Thyroid hormone receptor
Tris	Tris (hydroxymethyl) aminomethan
Trp	Treptophan
TSH	Thyroid-stimulating hormone
USF	Upstream stimulatory factor
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low density lipoprotein
VLFA	Very long chain fatty acids
ZNF	zinc finger protein

Contents *Page*

1- Introduction 1

1.1 Structure and function of ATP-binding cassette (ABC) transporters 1

1.1.1ABCA (ABC1) subfamily 2

1.1.1.1 ABCA1 3

1.1.2 ABCB (MDR/TAP) subfamily 8

1.1.3 ABCC (CFTR/MRP) subfamily 9

1.1.4 ABCD (ALD) subfamily 10

1.1.5 ABCE (OABP) and ABCF (GCN20) subfamilies 11

1.1.6 ABCG (white) subfamily 11

1.1.6.1 ABCG members in sterol homeostasis 12

1.1.7 ABC transporters in hepatobiliary transport 14

1.1.8 ABC transporters in macrophages 15

1.2 Steroid hormones 17

1.2.1 Estrogen receptors (ERs) 17

1.2.2 Sex hormones 19

1.2.3 Corticosteroid hormones 21

1.3 Non steroid hormones 23

1.3.1 Thyroxin 23

1.3.2 Leptin 24

1.3.3 Insulin and glucagons 25

1.3.4 Luteinizing hormone 28

1.3.5 Somatostatin 29

2- Aims of work 31

3- Material and methods 32

3.1 Cell culture 32

3.2 RNA isolation 32

3.3 Reverse transcription 33

3.4 Relative quantification by TaqMan^{TM} real time RT-PCR 33

3.5 Cholesterol and phospholipid effluxes 36

3.6 Western Blot 37

3.7 Data analysis 38

4- Results 39

4.1 Cholesterol and choline-phospholipid effluxes in human macrophages and HepG2 cells 39

4.2 ABC transporter gene expression in human macrophages 47

4.3 ABC transporter gene expression in HepG2 cells 49

4.4 Effect of insulin concentration on ABCA1 in human macrophages 55

4.5 Time kinetic of insulin on ABCA1, ABCG1 and ABCA7 genes expression in macrophages 56

4.6 Time kinetic of insulin on ABCA1 gene expression in HepG2 cells 58

4.7 Insulin stimulates ABCA1 expression via the MAP kinase pathway 59

4.8 Time kinetic of β-estradiol on ABCA1 and ABCG1 genes expression in human macrophages 61

4.9 Insulin effect on transcription factor gene expression 63

4.10 ERs mRNA expression in macrophages and HepG2 cells 65
5- Discussion 66
6- Summary 81
7- Reference List 83

1.Introduction

1.1 *Structure and function of ATP-binding cassette (ABC) transporters*

ABC transporter protein usually consists of two transmembrane domains (TMD) and two nucleotide binding domains (NBD) or ATP-binding cassettes (ABC). NBD is composed of two short, conserved peptides, the Walker A and Walker B motifs (Walker et al, 1982), which are required for ATP binding (Hyde et al, 1990). The signature motif is located between both Walker motifs and is characteristic for each ABC subfamily (Higgins et al, 1988). ABC transporters are either present in one polypeptide chain (fullsize transporter) or in two polypeptides (halfsize transporter), and several arrangements of the TMD and ABC motifs are found in human ABC proteins. TMD0-(TMD-ABC)2, which is one of the fullsize transporters, contains an additional five transmembrane spans in the N-terminal series of (ABC-TMD)2. (TMD-ABC)2 structures are represented in the ABCA, ABCB, and ABCC families, whereas the TMD0-(TMD-ABC)2 arrangement is solely present in specific members of the ABCC subfamily. The (ABC-TMD)2 is only found in yeast and not present in human ABC molecules. Halfsize transporters were either TMD-ABC organization, as in ABCD subfamily, or ABC-TMD, as in ABCG subfamily. In both cases, creation of a functional transporter requires the assembly as a homodimer or heterodimer. Most halfsize molecules are routed to intracellular membrane systems such as mitochondria, peroxisomes, the endoplasmic reticulum and the Golgi compartment (Klein et al, 1999). However ABCG2, a member of the ABCG subfamily, has been localized to the plasma membrane (Rocchi et al. 2000). ABCF1 is associated with ribosomes and interacts with eukaryotic initiation factor 2 (eIF2) and thereby plays a key role in the initiation of mRNA translation (Tzyack et al, 2000). ABC transporters can be split into two different sections depending on their mode of action. The active transporters or pumps, such as members of the ABCB subfamily, couple the hydrolsis of ATP and the resulting free energy is utilized for the movement of molecules across membranes against a chemical concentration gradient (Ueda et al, 1999). In contrast,

several ABC proteins which show nucleotide binding, have very low ATP hydrolysis. These molecules mainly function as transport facilitators and include ABCC7 (CFTR) (Szabo et al, 1999), ABCC8 (SUR1), ABCC9 (SUR2) (Bryan and Aguilar-Bryan, 1999), and ABCA1 (Szakacs et al, 2001).

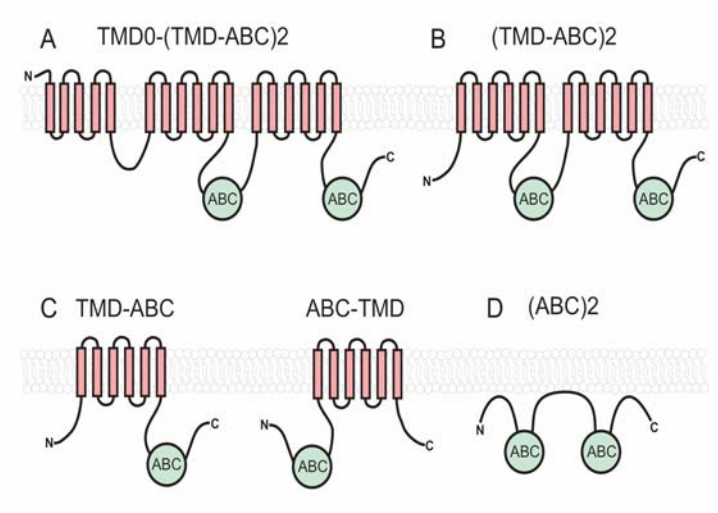

Fig.1. Diagram depicting domain arrangements of human ABC transporters.
The ATP-binding cassette (ABC) consists of Walker A and Walker B motifs, separated by the signature motif characteristic for each ABC transporter subfamily. The membrane spanning domains are depicted as barrels. **(A)** The TMD0-(TMD-ABC)2 structure of ABCC (MRP) family members is shown. In addition to the regular fullsize type, containing the (TMD-ABC)2 domain arrangement, this type displays an additional five transmembrane domains termed TMD0. **(B)** Prototype ABC transporter with the (TMD-ABC)2 structure. **(C)** Two alternative types of halfsize molecules, TMD-ABC and ABC-TMD. Only corresponding half-molecule organizations are able to form heterodimers. **(D)** The (ABC)2 type of molecules lacking transmembrane domains is unlikely to function as transporter. (Klein et al, 1999).

1.1.1 ABCA (ABC1) subfamily

The ABCA family is a fullsize transporter and ABCA1, ABCA4 (ABCR), and ABCA2 are the largest proteins with 2261, 2273, and 2436 amino acids, respectively. Most of the ABCA proteins are expressed at low levels and also predominantly in specific tissues, such as ABCA1 in macrophages and ABCA4 (ABCR) in photoreceptor cells (Allikmets, 2000). In contrast to all other ABC subgroups, the ABCA subfamily has no counterpart. Based on the genomic locations and phylogenetic analyses (Broccardo et al, 1999), two distinct divisions of ABCAs can be formed. The first group contains five genes located in a cluster on chromosome 17q24 (ABCA5, ABCA6, ABCA8, ABCA9, and ABCA10) and the second

group consists of seven genes distributed over six different chromosomes (ABCA1, ABCA2, ABCA3, ABCA4, ABCA7, ABCA12, ABCA13). The transcriptional control of at least seven ABCA members is controlled or influenced by lipids (Kaminski et al, 2001) indicating an important role of the whole ABCA subfamily in cellular lipid transport processes (Schmitz et al, 2000). ABCA4 is an active retinoid-PE-complex transporter which displays strong, lipid activated ATPase activity (Ahn and Molday, 2000). In addition to the high expression in neuronal tissues (Zhou et al, 2001), ABCA2 is also present in liver, kidney, and macrophages (Vulevic et al, 2001). ABCA2 was localized with endosomal/lysosomal markers and linked to the transport of sterols including retinoids, steroids and lipids (Paine and Flower, 2000). The ABCA7 protein is predominantly found in myelo-lymphatic tissues (Kaminski et al, 2000) and presumbly has a role in the development of hematopoietic cell lineage (Broccardo et al, 2001) and may be involved in the transport of phosphatidylserine and ceramide-species and thus be linked to apoptotic processes (Kielar et al, 2003). The ABCA3 protein is an integral part of the surfactant lamellar body membrane in lung alveolar type II cells (Yamano et al, 2001). Pulmonary surfactant is a complex of phospholipids, neutral lipids, and specific proteins. It is essential for a normal lung function, because it reduces surface tension at the air-liquid interface of alveolar spaces. Phospholipids comprise 80% of the mass of surfactant, of which 80-85% are phosphatidylcholines (PC). Increasing in ATP levels in bronchoalveolar lavage fluid is sufficient to stimulate surfactant secretion (Rice et al, 1989).

1.1.1.1 ABCA1

It is a 2261- amino acids integral membrane protein that is a member of a superfamily of ABC transporters that utilizes ATP as a source of energy for transporting lipids and other metabolites across membranes (Dean et al, 2001). ABCA1 comprises 2 halves of similar structure that are linked covalently. Each half has a nucleotide-binding domain (NBD) containing 2 conserved peptide motifs known as walker A and walker B, which are present in many proteins that utilize ATP, and a transmembrane domain containing six helixes. ABCA1

is predicted to have an N-terminus oriented into the cytosol and 2 large extracellular loops that are highly glycosylated and linked by one or more cysteine bonds (Bungert et al, 2001). ABCA1 either directly or indirectly mediates transport of cholesterol and phospholipids across cellular membranes, where they are removed from the cells by apolipoproteins. Its homology with other better characterized ABC transporters suggests that ABCA1 may form a channel in the membrane that promotes ”flopping” of lipids from the inner to the outer membrane leaflet by an ATPase dependent process (Oram and Lawn, 2001). ATPase activity in ABCA1 is not actually involved in lipid transport, so its function is considered as a regulator rather than an active transporter (Szakacs et al, 2001). ABCA1 localizes to the plasma membrane and intracellular compartments (Neufeld et al, 2001), where it could potentially facilitate transport of lipids to either cell surface-bound or internalized apoproteins. Thus ABCA1 removes cholesterol that will accumulate as cytosolic cholesterylester lipid droplets. Two models have been proposed to account for the ability of ABCA1 to target specific lipid domains. The exocytosis model implies that excess intracellular cholesterol is packaged into transport vesicles, or raft perhaps in the Golgi apparatus, which translocate to domains in the plasma membrane containing ABCA1 (Oram and Lawn, 2001). The retroendocytosis model suggests that ABCA1 and apolipoprotein-containing vesicles endocytose to intracellular lipid deposits, where ABCA1 mediates lipid transport into the vesicle lumen for release by exocytosis (Takahashi and Smith, 1999 and Santamarina-Fojo et al, 2001). The carboxy terminus has been reported to interact with β2-syntrophin and utrophin in macrophages (Buechler et al, 2002), forming a protein complex that might couple ABCA1 to the actin cytoskeleton. High intracellular cholesterol alters ions channels resulting in membrane polarization with subsequent increase intracellular Ca^{++}. Excess Ca^{++} possibly stimulates the dephosphorylation of β2-syntrophin with a subsequent release of ABCA1/cholesterol/phospholipid vesicles from the actin cytoskeleton (Murthy et al, 2002). ABCA1 is selectively expressed on the basolateral membranes of cultured intestinal

(Ohama et al, 2002), and hepatic cells (Neufeld et al, 2002), indicating the presence of factors that target ABCA1 to specific membranes in polarized cells.

Oram and co-workers, (2000) showed that incubation of macrophages with 8-Br-cAMP stimulated ABCA1 protein and mRNA with highest level achieved after 24 h incubation. Withdrawal of 8-Br-cAMP resulted in bringing the ABCA1 protein and mRNA to the basal level within 2–6 h, suggesting that ABCA1 protein is highly unstable and gets degraded in the absence of 8-Br-cAMP (Oram et al, 2000). Incubation of human and murine macrophages with lipoproteins, oxysterols, and oxidized LDL induced ABCA1 mRNA concomitant with increased levels of LXR-α expression (Venkateshwaran et al, 2000). Both RXR-α and LXR-α agonists induce ABCA1 mRNA expression. Repa and co-workers, (2002) identified the heterodimeric partner of LXR-α in the induction of ABCA1 mRNA by LXR-α agonists (Repa et al, 2002). They showed that both RXR-α and LXR-α agonists induce ABCA1 mRNA in duodenum, jejunum, ileum, and macrophage but not in the liver (Repa et al, 2002). RXR-α agonists, (Venkateshwaran et al, 2000), LXR-α agonists (Repa et al, 2002), and PPAR-α and PPAR-γ agonists (Chawla et al, 2001) induce the transcription of ABCA1. Addition of PPARs and LXR-α agonists showed additional influences on ABCA1 upregulation, suggesting that these agonists influence ABCA1 transcription via independent mechanism. PPAR-α and PPAR-γ receptors are nuclear receptors that heterodimerize with LXR-α to modulate the expression of target genes involved in lipid and glucose metabolism. The ligands for PPAR-α and PPAR-γ induce ABCA1 mRNA in primary human macrophages via LXR-α mediated pathway (Chinetti et al, 2001). Another PPAR receptor, PPAR-δ, expressed in many tissues, has been implicated in the upregulation of ABCA1 gene expression associated with increased plasma levels of HDL (Oliver et al, 2001). Treatments of macrophages with IFN-γ reduced ABCA1 mRNA and cholesterol efflux to apoA1 acceptor (Panousis et al, 2000), suggesting that IFN-γ may promote foam cell formation and accelerate the progression of atherosclerosis. Geranylgeranyl pyrophosphate, which is a major

metabolite in the mevalonate pathway, is a potent suppressor of ABCA1 by acting as an antagonist of LXR-α, and also by Rho-GTP binding proteins (Gan et al, 2001). Wang and Oram (2002) showed that unsaturated fatty acids inhibited ABCA1-mediated cholesterol efflux by enhancing the ABCA1 protein degradation but the saturated fatty acids, palmitate and stearate, inhibited neither ABCA1-mediated cholesterol and phospholipid effluxes nor ABCA1 protein expression (Wang and Oram, 2002). However, the exact mechanism of triggering the ABCA1 degradation by fatty acids is still not known, but the mechanism appears to be different than that observed with the cAMP withdrawal (Oram et al, 2000). In addition to functional regulatory domains in the exon, an LXR-α element was recently reported in the intron 1 sequences (Singaraja et al, 2001). The ABCA1 promoter, in addition to TATA box and CAAT box, also contains other potential regulatory sites. Direct repeat of the nuclear receptor half site TGACCT spaced by 4 nucleotides (DR4) binds to the LXR/RXR heterodimers, and mutation in the DR4 abolished the oxysterol-responsive ABCA1 activation, suggesting that DR4 is important in LXR/RXR-mediated upregulation of ABCA1, as well as for oxysterol-induced activation of ABCA1 (Repa et al, 2002). The E-Box motif located –147 bp upstream is an important ABCA1 promotor activation that binds to transcriptional factors USF1 and USF2. It also binds to the transcriptional repressor Fra2 (Yang et al, 2002). cAMP increased ABCA1 mRNA but did not not involve ABCA1 mRNA stability (Oram et al, 2000). This implies that a cAMP response element should be present in the ABCA1 promoter but has not been definitively identified. The transcriptional repressor ZNF202 was found to be associated with Downregulation of ABCA1 through binding with the GnT motif of ABCA1 promoter and mediates the transcriptional repression (Porsch-Ozcurumez et al, 2001).

The major clue that ABCA1 is involved in cellular cholesterol removal and lipid efflux was the identification of mutations in the human gene as the defect in familial HDL-deficiency syndromes such as classical Tangier disease (TD; Bodyioch et al, 1999). The most striking feature of these patients is the almost complete absence of plasma HDL, low serum

cholesterol levels and a markedly reduced effluxes of both cholesterol and phospholipids from the cells (Bottcher et al, 2000). Plasma HDL from TD patients is composed of small pre-β1-migrating HDL particles containing solely apoA-I and phospholipids but lack free cholesterol and apoA-II (Asztalos et al, 2001). The low HDL-levels seen in Tangier disease are mainly due to an enhanced catabolism of HDL precursors (Asztalos et al, 2001). In TD patients, neither cholesterol absorption nor its metabolism is significantly affected. However, the concentration of LDL-cholesterol is only 40% of healthy controls and the particles are often enriched in triglycerides. The reduction in LDL levels is mainly caused by disturbance of the cholesterol ester transfer pathway resulting in changes of LDL composition and size (Schaefer et al, 2001). Heterozygotes ABCA1 mutations (FHD) have approximately 50% of plasma HDL, but normal LDL levels (Tall and Wang, 2000) and more than three-fold risk to develop coronary artery disease in affected family members and earlier onset compared to unaffected members (Clee et al, 2001). In addition, accumulation of cholesteryl esters either in the cells of the reticuloendothelial system (RES) or in the vascular wall was leading to splenomegaly, enlargement of tonsils and lymph nodes, or premature atherosclerosis (Schmitz et al, 2000).

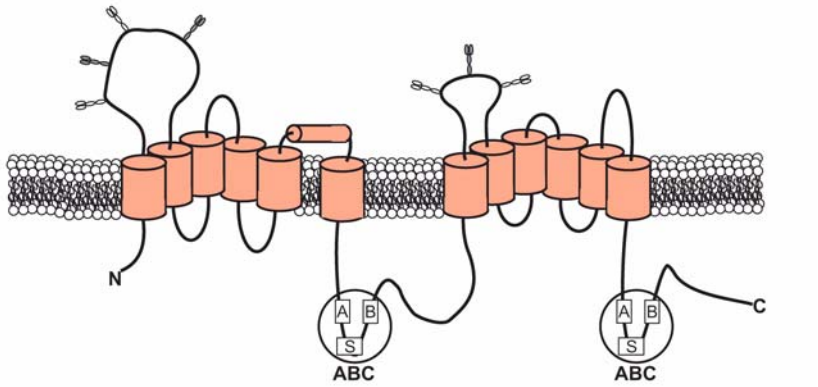

Fig.2. The predicted structure of ABCA1.
ATP-binding cassette A1 (ABCA1) transporter is 2,261-amino acids integral membrane protein. ABC transporters are defined by the presence of nucleotide-binding domains containing two conserved peptide motifs known as Walker A and Walker B that are present in many proteins that utilize ATP as a source of energy and a unique amino acid signature between the two Walker motifs called signature domain (S) which is a highly hydrophobic segment and defines the family. ABC transporters are integrated into the membrane by domains containing six transmembrane helices (Langmann et al, 1999).

1.1.2 ABCB (MDR/TAP) subfamily

ABCB1 (MDR1) has the ability to mediate multidrug resistance in cancer cells and is localized to the apical membrane of polarized cells and the major sites of expression are found in the liver, the intestine and the blood-brain barrier and one proposed physiological function of MDR1 is the protection of cells by extruding lipophilic cytotoxic drugs (Pastan and Gottesman, 1991). In addition ABCB1 can transport a variety of lipids: PC analogs, phosphatidyl-ethanolamine (PE), sphingomyelin (SM), cholesterol and glucosylceramide (GlcCer) (Van Helvoort et al, 1996). So, it has been suggested that the transport of cytotoxic drugs, which are mostly lipophilic, is coupled to the translocation of cholesterol and phosphocholine (Lavie etal, 2001). ABCB1 is also involved in the secretion of platelet-activating factor (PAF) (Raggers et al, 2001). An unexpected role of ABCB1 in the immune response has been recently identified in mice (mdr1a-/- mice) (Panwala et al, 1998). ABCB1 can cotransport apoE and β-amyloid and thereby may contribute to the aetiology of Alzheimer's disease (Maggio et al, 2002). Two halfsize members of the subfamily, ABCB2 (TAP1) and ABCB3 (TAP2) are transporters associated with tissue antigen presentation (TAP) and form a functional heterodimer to transport peptides from the cytoplasm into the endoplasmic reticulum from where the presentation of peptide antigens via major histocompatibility complex (MHC)-I will start (Herz and Beffert, 2000). ABCB9, which is closely related to ABCB2 and ABCB3, has been mainly found in lysosomes (Zhang et al, 2000). Although ABCB9 has been proposed to be involved in TAP-dependent processes, its exact function is currently unknown. The remaining four ABCB proteins (ABCB6, ABCB7, ABCB8, and ABCB10) are all targeted to the inner mitochondrial membrane and play a role in cellular iron homeostasis by transporting iron-sulfur (Fe/S) cluster precursor proteins (Zhang et al, 2000). In this respect, a mutation in ABCB7, which is located on the X-chromosome, has been linked to X-linked sideroblastic anaemia and ataxia (XLSA/A) (Allikmets et al, 1999).

1.1.3 ABCC (CFTR/MRP) subfamily

Subgroup of ABCC family can be distinguished by the presence of a TMD0-(TMD-ABC)2 domain arrangement (ABCC1, ABCC2, ABCC3, ABCC6, ABCC8, ABCC9, ABCC10), whereas the other proteins in this subfamily exhibit the (TMD-ABC)2 structure. Although the TMD0 part is not required for transport activity, but it is essential for a proper ABCC1 function (Bakos et al, 1998). Among the (TMD-ABC)2 molecules, ABCC7 (CFTR), which is characterized by an extraordinary domain structure and contains a regulatory domain, is controlled by cAMP and thereby enables ATP binding and hydrolysis at the nucleotide binding domains. It is in turn a control opening and closing of the chloride channels (Sheppard and Welsh, 1999). Mutations in ABCC7 (CFTR) cause cystic fibrosis by affecting numerous secretion processes. ABCC1, ABCC2, and ABCC3 are all able to transport anti-cancer drugs. ABCC2 (MRP2) which is located in the apical membrane of polarized epithelial cells and particularly to the canalicular membrane of hepatocytes, appears to participate in the hepatobiliary secretion of organic anions and has therefore originally called CMOAT (Keppler and Konig, 2000). ABCC3 (MRP3) is also an organic ion transporter (Hirohashi et al, 1999). ABCC4 and ABCC5 have been shown to function as cellular efflux pumps for anti-human immunodeficiency virus drugs such as adefovir (PMEA; Schuetz et al, 1999) and cancer chemotherapy (e.g. 6-mercaptopurine and thioguanine) (Borst et al, 2000). The physiological role as of ABCC6 (MRP6) is still unclear (Kool et al, 1999) and mutations in that gene have been detected in the connective tissue disorder pseudoxanthoma elasticum (PXE) (Le Saux et al, 2000). ABCC6 is highly expressed in liver and kidney cells and it may transport or remove toxic metabolites which destroy connective tissue cells (Dean et al, 2001). ABCC8 (SUR1) and ABCC9 (SUR2) bind sulfonylurea with high affinity and interact with potassium inward rectifiers KIR6.1 and KIR6.2, to form a large octameric channel (SUR/KIR6.x)4 (Forestier et al, 2003). These heteromeric channels regulate insulin release in response to glucose metabolism and sulfonylureas which are widely used to stimulate insulin

secretion in type 2 diabetic patients because they close these ATP-sensitive potassium (K_{ATP}) channels in the pancreatic beta-cell membrane (Bryan and Aguilar, 1999). ABCC11 and ABCC12 are found duplicated on chromosome 16q12 (Tammur et al, 2001), and are mapped to a region harboring gene(s) for paroxysmal kinesigenic choreoathetosis, a disease which is characterized by recurrent and brief attacks of involuntary movements induced by sudden voluntary movements (Lee et al, 1998).

1.1.4 ABCD (ALD) subfamily

This subfamily is composed of four peroxisomal half-size ABC transporters and is involved in very long fatty acid (VLFA) transport. Mutations in ABCD1 and ABCD3 are associated with adrenoleukodystrophy (ALD) and Zellweger syndrome 2 (ZWS2), respectively (Mosser et al, 1993). The transcripitonal regulation of ABCD genes was done by lipids and there is a strong evidence that nuclear hormone receptor ligands, especially RXR ligands and PPAR ligands induce the ABCD1 promoter (Fourcade et al, 2001).

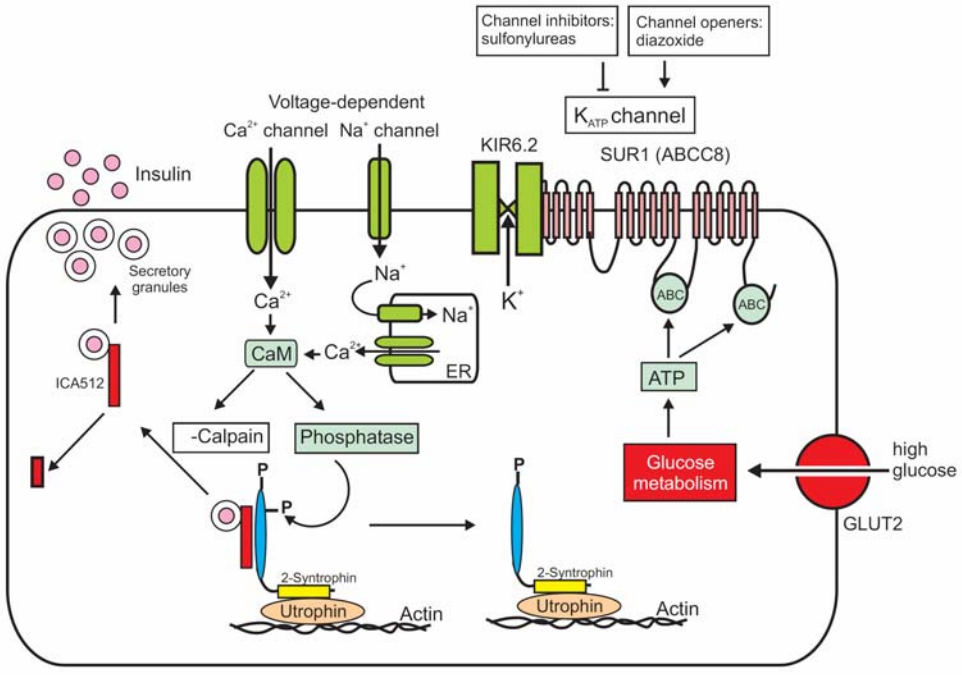

Fig.3. Schematic model for K_{ATP} channel controlled insulin secretion from pancreatic β-cells.
Entry and metabolism of glucose into pancreatic β-cells leads to increased levels of intracellular ATP. This increase causes binding of ATP to the nucleotide binding domains of ABCC8 (SUR1) and to KIR6.2. Thereby, the K_{ATP}-channel closes and the plasma membrane is depolarized. The opening of voltage-gated Ca^{2+} channels and voltage-dependent Na^+

channels raises the intracellular Ca^{2+} concentration by Ca^{2+} influx and mobilization of intracellular Ca^{2+} stores, respectively. The increased level of intracellular Ca^{2+} stimulates the dephosphorylation of β2-syntrophin and the dissociation of β2-syntrophin-utrophin-actin complexes from ICA 512 and secretory granules. Following dissociation of β2-syntrophin, ICA 512 is cleaved by Ca^{2+}/calmodulin (CaM) activated calpain, resulting in the mobilization of secretory granules from the cytomatrix and exocytosis of insulin. The pancreatic K_{ATP}-channels are also regulated by important therapeutic pharmacological agents, such as sulfonylureas which are widely used in the treatment of non insulin dependent diabetes, stimulate insulin secretion by closing the K_{ATP}-channels (Aguilar-Bryan et al, 1998).

1.1.5 ABCE (OABP) and ABCF (GCN20) subfamilies

This subfamily contains four half-size ABC transporters, which are ubiquitously expressed in human tissues and do not possess transmembrane domains. The ABCE1 gene encodes an oligoadenylate binding protein (OABP), which seems to participate in innate immune defence (Bisbal et al, 2001). Oligoadenylates, which are produced from virus-infected cells, are activators of RNaseL that in turn degrades cellular RNAs and thereby blocks protein synthesis in infected cells. ABCE1 binds these oligonucletides and thus inhibits RNAseL, which implies that ABCE1 is involved in the control of immune reactions. ABCF1 shares some interesting features with ABCE1. Thus, ABCF1 is involved in the control of protein synthesis and also in the control of the immune system. ABCF1 binds to the translation elongation initiation factor 2 (eIF2) and seems to modulate its phosphorylation state (Lee et al, 1998). In addition, ABCF1 has been copurifed with ribosomal components confirming its role in protein translation (Tzyack et al, 2000). Richard and colleagues (1998) identified ABCF1 as a TNFα-induced transcript in synoviocytes (Richard et al, 1998). They suggest that this ABC protein could be part of inflammatory processes related to rheumatoid arthritis (Richard et al, 1998).

1.1.6 ABCG (white) subfamily

The human white or ABCG subfamily consists of five genes (ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8) and one gene so far only found in rodents (ABCG3) (Schmitz et al, 2001). The ABCGs are intended to dimerize to form active membrane transporters. Among the half-size molecules ABCG proteins have a peculiar domain organization characterized by

a nucleotide binding domain (ATP-binding cassette) at the N-terminus followed by six transmembrane spanning domains. ABCG1 was described as the human homologue of the Drosophila white gene (Croop et al, 1997). Earlier indications linked ABCG1 with the congenital recessive deafness (DFNB10) syndrome using its chromosomal localization on chromosome 21q22.3 (Bonne et al, 1996). ABCG1 along with five other known genes had been reported as candidates for DFNB10 (Berry etal, 2000). Also, G2457A polymorphism in the ABCG1 mRNA is associated with mood and panic disorders and related to suicidal behavior (Rujescu et al, 2000). The most interesting report dealing with ABCG1 function came from a report by Klucken and colleges, (2000), which identified ABCG1 as a sterol induced gene that participates in cholesterol and phospholipid effluxes, especially in macrophages and foam cells (Klucken et al, 2000). The ABCG2 protein has been shown to be amplified and overexpressed in human cancer cells and is capable of mediating drug resistance (Miyake et al, 1999). In contrast to most other halfsize ABC transporters, the bulk of the ABCG2 protein has been localized to the plasma membrane, with a minor fraction found within intracellular membranes (Rocchi et al, 2000). ABCG5 and ABCG8 had been identified and linked to the human disease β-Sitosterolemia (Lee et al, 2001). ABCG4 transporter was identified as a sterol-sensitive gene (Engel et al, 2001).

1.1.6.1 ABCG members in sterol homeostasis

ABCG1 was identified as a target gene involved in macrophage lipid homeostasis (Klucken et al, 2000). Like ABCA1, ABCG1 is upregulated during the differentiation process of monocytes into mature macrophages and is strongly induced by foam cell conversion of these macrophages (Langmann et al, 1999). HDL_3 as the cholesterol acceptor suppresses ABCG1 mRNA and protein expression (Klucken et al, 2000). ABCG1 was upregulated by modified LDL (Venkateswaren et al, 2000). TNFα or LPS has no impaction on ABCG1 mRNA expression (Venkateswaren et al, 2000). Some oxysterols and RXR-specific ligands can upregulate ABCG1 expression via the LXR/RXR pathway. There are at least three

independent ABCG1 promoters. Whereas the activity of promoter 1 has not been proven (Lorkowski et al, 2001), promoter 3 of ABCG1 has been shown to bind the transcription factors LXR/RXR and thereby mediate the sterol-dependent induction of the gene (Kennedy et al, 2001) and promoter 2 was described as an independent inhibitory site of the transcriptional repressor ZNF202 (Porsch-Ozcurumez et al, 2001). The residual phospholipid and cholesterol effluxes present in cells from patients with Tangier disease along with a compensatory upregulation of ABCG1 in these cells supporting the function of ABCG1 in intracellular mobilization of lipid stores (Lorkowski et al, 2001). ABCG1 was localized in intracellular compartments mainly associated with the ER and Golgi-membranes (Klucken et al, 2000). ABCG5 and ABCG8 implicated in the efflux of dietary sterols mainly plant sterols like sitosterol and shell fish sterols from intestinal epithelial cells back into the gut lumen and from the liver to the bile duct (Salen et al, 1970). β-Sitosterolemia (phytosterolemia or shellfishsterolemia) was a rare autosomal recessive disorder first described by Bhattacharyya and Connor in 1974 (Bhattacharyya and Connor, 1974). The disease is characterized by enhanced trapping of cholesterol and other sterols, including plant and shellfish sterols, within the intestinal cells and the inability to concentrate these sterols in the bile with strongly increased plasma levels of plant sterols e.g. β-sitosterol, campesterol, stigmasterol and avenosterol whereas total sterol levels remain normal or just moderately elevated (Salen et al, 1992). Patients suffer from tendon and tuberous xanthomas at an early age, premature development of atherosclerosis and coronary artery disease. In some cases hemolytic episodes, hypersplenism, platelet abnormalities, arthralgias and arthritis have been described (Bjorkhem, and Boberg, 1999). There are several mutations and a number of polymorphisms have been identified in ABCG5 and ABCG8 (Lee et al, 2001). β-Sitosterolemia occurs due to either mutation in ABCG5 or ABCG8, but never in both genes together (Lee et al, 2001). Dietary sterols including cholesterol and plant sterols which enter the intestinal epithelial cells via micellar transport are released along the lysosomal route. β-Sitosterol and other plant

sterols are directly transported back to the gut lumen by the heterdimeric ABCG5/ABCG8 complex. The retained sterols are routed in the ER and either stored as cholesteryl esters in lipid droplets or alternatively packed into chylomicrons for further transport back to the liver. In the liver, the sterols are either transported to the peripheral tissues by VLDL and LDL particles or converted to bile acids which also mediated by ABCG5 and ABCG8. In addition to ABCG5 and ABCG8, other ABC transporters including ABCG1 and ABCA1 may also participate in intestinal sterol absorption mechanisms (Orso et al, 2000).

1.1.7 ABC transporters in hepatobiliary transport

Translocation of compounds from hepatocytes into the bile has been involved by ABC transporters localized in the hepatocyte apical (canalicular) membrane (Muller and Jansen 1998). These ABC proteins belong to the ABCB (MDR) and ABCC (MRP) subfamilies. The level of expression of ABCB1 (MDR1) in normal human liver (Silverman and Schrenk, 1997) will protect hepatocytes against harmful substances as xenobiotics, neurotoxins, and chemotherapeutics by active translocation into the bile (Smit et al, 1998). ABCB4 which is exclusively expressed in the liver apical membrane is a bile canalicular phosphatidylcholine translocase. Mutations in the human ABCB4 (MDR3) gene cause progressive familial intrahepatic cholestasis (PFIC) type 3 (De Vree et al, 1998). The third member of the ABCB subfamily involved in hepatobiliary secretion is ABCB11 which is the major, if not the only bile salt transporter of mammalian liver, hence the name bile salt export pump (BSEP). ABCB11 (BSEP) gene is mutated in patients with (PFIC) type 2 (Strautnieks et al, 1998). In the ABCC (MRP) subfamily, at least four members have been shown to be expressed in liver cells (Borst et al, 2000). In contrast, ABCC2 (MRP2) is highly expressed at the apical membrane domain, but ABCC1 (MRP1) is highly expressed at the basolateral membrane domain in normal liver (Borst et al, 2000). ABCC1 and ABCC2 comprise glutathione-conjugates (e.g. leukotriene C4), estrogen-and bilirubin-glucuronides, taurolithocholate-3-sulfate, and glutathione disulfide (GSSG). ABCC2 seems to be the major transporter of

anionic conjugates and hereditary defects of ABCC2 in humans cause the Dubin-Johnson syndrome (Kartenbeck et al, 1996). ABCC3 (MRP3) which has been localized to the basolateral membrane of hepatocytes can translocat the conjugated Glucuronate- and sulfate from blood sinusoids to hepatocyte but ABCC2 at the apical site can translocat them to the bile (Konig et al, 1999). ABCC6 (MRP6) has been localized to the lateral hepatocyte membrane but its physiological effect is not known (Madon et al, 2000).

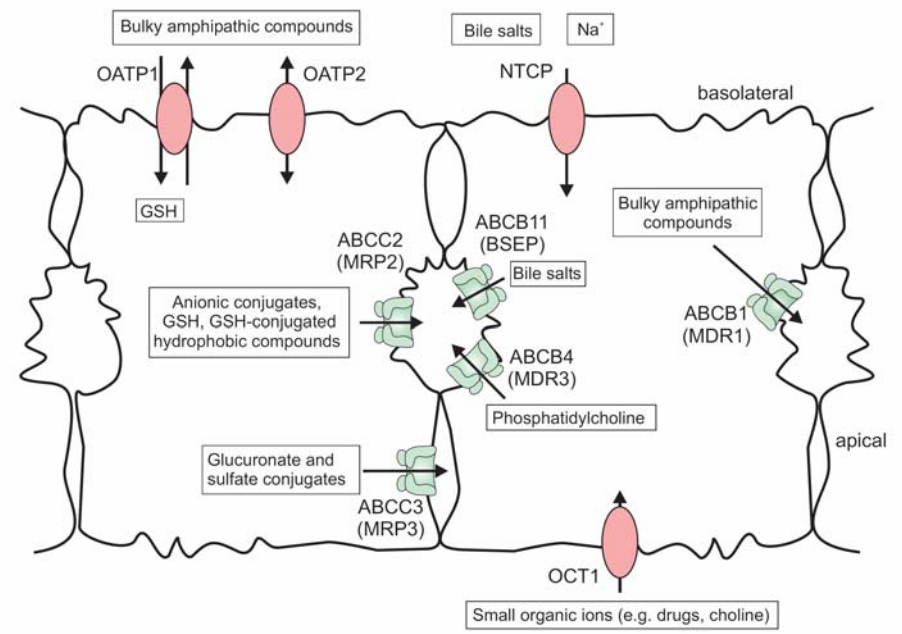

Fig.4. Overview of lipid transport proteins in hepatocytes.
Monovalent bile salts, such as taurocholate, are taken up into hepatocytes by the sodium-taurocholate cotransporting polypeptide (NTCP). The organic anion transporting polypeptides 1 and 2 (OATP1-2) are responsible for the uptake of bulky organic compounds, including bile salts and other organic anions, uncharged cardiac glycosides, and steroid hormones. Small, type 1 organic cations are transported by the organic cation transporter OCT1. Several ABC proteins belonging to the ABCB (MDR) subfamily or ABCC (MRP) subfamily are expressed in liver. ABCB1 (MDR1) is responsible for the excretion of bulky amphiphatic compounds into bile, whereas ABCB4 is a phosphatidylcholine translocase. Monovalent bile salts are secreted into the bile canaliculi by ABCB11. ABCC2 functions as a multispecific organic anion transport protein in the canalicular membrane. ABCC1 expressed in the basolateral membrane in normal hepatocytes, has similar substrate specificity to ABCC2. ABCC3 preferentially translocates conjugates with glucuronate or sulfate, whereas the physiological substrates for ABCC6 (MRP6) are unknown (Muller and Jansen, 1998).

1.1.8 ABC transporters in macrophages

The ABCA1 upstream region contains a macrophage specific promoter preceding exon 1. This sequence binds the repressors ZNF202 and USF1/2, as well as the activating factors Sp1/Sp3 and the oxysterol-induced RXR/LXR heterodimer (Langmann et al, 2002). A second

promoter located downstream of exon 1 has been recently implicated in the liver/steroidogenic expression of ABCA1 (Cavelier et al, 2001). The LXR/RXR responsive elements in promoter 1 were triggered by retinoic acid and oxysterol resulting in activation of the ABCA1 promoter and thereby induction of ABCA1 during lipid loading of macrophages. The most likely endogenous ligand for LXR-α is 27-hydroxycholesterol. Overexpression of cytochrome P27 (CYP27) in HepG2 cells leads to an increase in bile acid synthesis with a compensatory stimulation of cholesterol synthesis by increased HMG-CoA reductase (HMG-CoA R) activity but in extrahepatic cells, CYP27 overexpression results in an increase in intracellular 27-hydroxycholesterol leading to downregulation of HMG-CoA reductase and cholesterol synthesis (Hall et al, 2001). Cytochrome P27 (CYP27) deficient cells are not able to upregulate ABCA1 in response to sterols and since overexpression of CYP27 activates LXR/RXR (Fu et al, 2001). The earlier described LXR-ligands, 20(S)-hydroxycholesterol and 22(R)-hydroxycholesterol, are not present in cholesterol-loaded macrophages rendering them unlikely to be natural ligands of LXR (Fu et al, 2001). Zinc finger transcription factor ZNF202 is a transcriptional repressor of ABCA1 gene expression, which also prevents the induction of the gene by oxysterols by recruiting the universal corepressor KAP1 (Porsch-Ozcurumez et al, 2001).

Under disease conditions such as DM where the cells have low glucose levels low ATP levels and associated low HDL cholesterol levels, excessive mitochondrial energy production could induce mitochondrial exhaustment. This may result in cellular ATP shortage, a process that likely enhances the programmed cell death of lesion macrophages (Laffel, 1999). Mitochondrial exhaustment may also inhibit mitochondrial 27-OH sterol synthesis and its export from the mitochondrion, a critical pathway for LXR activation in response to cellular cholesterol stress (Fu et al, 2001). Since deficiency of 27-OH sterol which is observed in macrophage-derived foam cells and atherosclerostic lesions (Brown and Jessup, 1999), may be engaged in the pathophysiological mechanism of atherosclerosis. In light of these

complexities, prolonged or excess treatment with LXR agonists bears the potential risk of inducing mitochondrial failure and pro-apoptotic influences and may thus negatively affect lesion formation. ABCA1 is detectable not only on the plasma membrane but also in the cytosol and Golgi compartment of unstimulated fibroblasts; this may raise the possibility that ABCA1 could be a constituent of a vesicular transport route for lipids. ABCA1 acts as a translocator of lipids between the inner and outer plasma membrane (Lawn et al, 1999). ATP turnover of ABCA1 occurs at a very low rate whereas nucleotide binding induces conformational changes (Szakacs et al, 2001). ABCA1 acts as a facilitator of cholesterol/ phospholipids export within the cellular lipid export machinery rather than active pump function (Szakacs et al, 2001).

1.2 Steroid hormones

1.2.1 Estrogen receptors (ERs)

Estrogen receptors are members of the nuclear steroid receptor family, a large group currently totally approximately 150 different proteins, which are bound by their respective ligands and function as transcription factors in many different species including both invertebrates and vertebrates. The nuclear receptors are characterized by a highly conserved DNA binding domain and a moderately conserved ligand binding domain which also functions in transcriptional activation (Mangelsdorf et al, 1995). ERs are not only expressed in sex accessory tissues but also in many other types of cells including liver, bone, pituitary and cardiovascular cells.

The classical ER (now called ERα) contains 595 amino acids with a central DNA-binding domain (DBD), along with a carboxy-terminal hormone-binding domain (Jafrati et al, 1997). ERβ is somewhat shorter than ERα, containing 530 amino acids (Ogawa et al, 1998). ERα is essential for uterine growth and mammary gland development but is not essential for mediating the atherosclerotic inhibitory influences of estrogens in vascular injury (Jafrati et al, 1997). Thus, it is possible that ERα and ERβ have distinct functions in some tissues but not in

others. The region of highest homology between ERα and ERβ is in the DBD (95%). ERβ lacks a large portion of the carboxy-terminal F domain is important for the agonist impact of certain antiestrogens, such as tamoxifen (Montano et al, 1995). ER-mediated gene transcription is stimulated through at least two distinct transactivation domains located in the amino-terminal A/B region (called AF-1) and the carboxy-terminal E region of the receptor (called AF-2) (Kumar et al, 1987). The AF-1 domain is hormone-independent, whereas the AF-2 domain is hormone-dependent (Webster et al, 1989). Both AF-1 and AF-2 are required for maximal ER transcriptional activity and can function independently (Tzukerman et al, 1994). The activity of the AF-1 region of ERβ is negligible compared with the AF-1 of ERα (Cowley and Parker, 1999). Thus the activity of ERα may exceed that of ERβ on estrogen responsive element containing genes that require both transactivation domains. Antiestrogens such as tamoxifen require only the AF-2 domain for ER-mediated transcriptional activity but ER AF-1 can function as a partial estrogen agonist of tamoxifen action (McDonnell et al, 1995). After hormone binding and dimerization, ERs bind to DNA with high affinity through their DBD (C region) at specific sites and termed estrogen responsive elements in the promoter region of target genes to alter gene transactivation so, ERs act directly as transcription factors (Kumar and Chambon, 1988). In the absence of estrogen, ERs exist as inactive oligomeric complexes with a number of other proteins including chaperon proteins, namely the heat shock proteins Hsp90 and Hsp70 and cyclophilin-40 and p23 (Pratt and Toft, 1997). The role of Hsp90 and other chaperons may be to maintain the receptors folded in an appropriate conformation to respond rapidly to hormonal signals. Following hormone binding, the oligomeric complex dissociates allowing the receptors to function directly as transcription factors by binding to DNA (Kumar and Chambon, 1988). Members of the epidermal growth factor family of tyrosine kinase receptors and insulin-like growth factor (IGF) can activate ER by direct phosphorylation (Kato et al, 1995). Different ligands can

interact with ER and enhance ER transcriptional activity (coactivators), or decrease its activity (corepressors) (Kamei et al, 1996).

1.2.2 Sex hormones

Atherosclerotic cardiovascular disease is the leading cause of mortality among postmenopausal women. Abnormalities in lipid and lipoprotein metabolism (eg, increased LDL and decreased HDL levels) commonly seen in post-menopausal women have been attributed to the increased coronary heart disease related mortality in these individuals (Sacks and Walsh, 1994). Significant decreases in HDL cholesterol and increases in LDL cholesterol, triglycerides and Lp(a) which is a new marker of cardiovascular risk were affected by an increasing of age and increasing time since menopause (Godsland, 2001). The use of estrogen as hormone replacement therapy (HRT) in postmenopausal women was associated with increase triglycerides production (Walsh et al, 1991), increase lipoprotein lipase activity (Tilly-Kiesi et al, 1997), and reduction of Lp (a) levels (Tuck et al, 1997). Because the decline in estrogen levels is the primary metabolic alteration observed in postmenopausal women, it has been thought that endogenous concentrations of estrogen may have fundamental roles in lipoprotein mediated development of atherosclerotic coronary heart disease. Clinical studies have indicated that estrogen therapy significantly elevated plasma HDL levels and decreased LDL concentrations, suggesting a favourable influence on the plasma lipoprotein profile (Lobb, 1991). In postmenopausal women under estrogen therapy have a lower relative risk of coronary event than postmenopausal women who are not on estrogen therapy (Grady el al, 1992). The favorable alterations in HDL levels appear to be a well-established that estrogen can prevent atherosclerotic cardiovascular disease. However, the mechanism by which estrogen raises HDL levels is not clearly understood and it may be due to increase production rate of HDL-protein and apoA-I (Walsh el al, 1994). Contrary to these observations, it was shown that the treatment of premenopausal women with estradiol resulted in decreased hepatic lipase activity and suggested that estrogen may increase HDL level by decreasing the

rate of HDL catabolism, which has been thought (but not proven) to be mediated via this enzyme (Tikkanen el al, 1982). Srivastava (2002) found that estrogen's antiatherogenic effects may occur via ABCA1-mediated pathway and circulating HDL levels may influence expression of ABCA1 in mice (Srivastava, 2002). Postmenopausal women with hypercholesterolemia, use of combined oral estrogen and progesterone therapy can result in a more cardioprotective lipoprotein-lipid profile than that achieved with either therapy used alone (Darling et al, 1999). Regimens containing norethindrone acetate as the progestin not only attenuate the influences of estrogen on triglyceride levels but also attenuate the increase in HDL-C levels seen with estrogen alone (Lee and Shulman, 2002).

Little is known about the atherogenic potential of testosterone which has frequently been made responsible for the gender difference in the onset of coronary heart disease. For over the last four decades, it had been hypothesized that androgen may play a role in preventing the development of atherosclerosis and coronary artery disease (Khaw, 1996). In recent years, there has been a surge of public interest in androgen because of its reported anti-atherosclerotic and anti-aging. In clinical studies, testosterone was found to exert both beneficial and adverse effects on cardiovascular risk factors and vascular function. The increasing use of testosterone for treatment of male hypogonadism, as a hormone replacement therapy for aging men, and its use in male contraception make the issue important of whether exogenous testosterone is pro- or antiatherogenic (Von Eckardstein, 1998). The major argument for the putative atherogenicity of testosterone is its lowering the high density lipoprotein HDL-cholesterol (Alexandersen et al, 1996). Numerous clinical and epidemiological studies have demonstrated the inverse association between HDL cholesterol and the risk of coronary heart disease events (Gordon and Rifkind, 1989). Men have considerably lower levels of HDL cholesterol than women. Moreover, application of exogenous testosterone leads to a dose-dependent decrease of HDL cholesterol, whereas either surgical or chemical castration causes a significant increase of HDL cholesterol in men

(Whitsel et al, 2001). Testosterone led to a dose dependent up-regulation of SR-BI mRNA and the protein levels and consequently increased HDL3-induced cholesterol efflux from macrophages (Langer et al, 2002) but not with Dehydroepiandrosterone (DHEA; Martin et al, 2003). DHEA and its sulfate ester (DHEAS) are sex hormone precursors of mainly adrenal origin, with weak androgenic action. DHEA and DHEAS are the most abundant steroids in the circulation, yet their biologic significance is unknown. A supraphysiological dose of testosterone can increase the expression of hepatic lipase (HL) in HepG2 cells (Langer et al, 2002). Moreover, testosterone had no influence on the expression of apoA-I in HepG2 cells and ABCA1 in either HepG2 cells or macrophages and these suggest that testosterone, despite lowering HDL cholesterol, intensifies reverse cholesterol transport and thereby exerts an anti-atherogenic rather than a pro-atherogenic (Langer et al, 2002). Others suggested that androgen was an atherogenic hormone by increasing human foam cell formation through elevating expression of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) but without significant impaction on LDL or scavenger receptor expression (Martin et al, 2003). Not only androgens but also estrogen, and progesterone exert gender-specific effects on human macrophage foam cell formation (Ng et al, 2001).

1.2.3 Corticosteroids

Actions of corticosteroids may be mediated by intracellular glucocorticoid receptors. These receptors are members of nuclear steroid hormone receptor superfamily related proteins (Mangelsdorf et al, 1995). These receptors share two highly conserved domains: a region of approximately 70 amino acids forming two zinc-binding domains, termed zinc fingers, which are essential for the interaction of the receptor with specific DNA sequences, and a region at the carboxy terminus that interacts with ligand (ligand-binding domain). Glucocorticoids enter cells and interact with the glucocorticoid receptor to change the GR conformation, induce GR nuclear translocation and activate transcription of target genes (Mangelsdorf et al, 1995). Dexamethasone is a synthetic form of glucocorticoid hormone but has high anti-inflamatory

potency, long duration of action ($t_{1/2}$ 36-72 h.), relative high affinity for GR than hydrocortisone (7.1:1 respectively) but no Na^+ retension potency.

The mechanisms of glucocorticoids action on atherogenesis are poorly understood. Glucocorticoids are able to decrease the expression of hepatic LDL receptors (Brindley and Salter, 1991), stimulate the net synthesis of apoB-100 and apoB-48 and also decrease their intracellular degradation (Wang et al, 1995). These changes are potentially atherogenic and the involvement of glucocorticoids in atherogenesis is supported by the strong correlation between increased serum cortisol in human and the extent of coronary artery disease (Bridley et al, 1993). Intracellular cholesterol movement in human SMC is also under glucocorticoid control by an increase in the flux of plasma membrane-located cholesterol into cells, promotion of cholesterol esterification and reduction of HDL_3-mediated cholesterol efflux (Petrichenko et al, 1997). Glucocorticoid treatment has been reported to markedly inhibit cholesterol synthesis in various tissues (cultured human fibroblasts, HeLa cells, lymphocytes), presumably through the inhibition of both HMG-CoA reductase and synthase activities (Lehoux et al, 1989). The ability of glucocorticoids to modulate the cholesterol synthesis and stimulate cholesteryl ester formation in SMC by rising in ACAT activity was no longer visible at 10^{-7} mol/L and increasing abruptly with increasing concentration up to 10^{-5} mol/L (Picard et al, 1981). Glucocorticoids, in turn, not only have been shown to inhibit cAMP production in rat SMC (Ito et al, 1994) but also directly regulate the HSL mRNA level in adipose tissue (Slavin et al, 1994). Glucocorticoids including Dexamethasone increase HSL activity by an increase in HSL mRNA levels (Slavin et al, 1994), this may have petential clinical significance in excess glucocorticoidscause the development of central (abdominal) adiposity due excess lipolysis of omental adipose tissue (Desprts, 1990), along with clinical sequellae such as insulin resistance, hypertension and hyperlipidemia (Desprts et al, 1990) . When glucocorticoid applied as anti-inflammatory drugs at high pharmacological doses in experimental animals, glucocorticoids seem to suppress the development of atherosclerosis,

despite enhancement of hypertriglyceridemia and hypercholesterolemia (Naito et al, 1992). Glucocorticoids were shown to inhibit leukocyte accumulation in the rabbit carotid artery and its intimal thickening (Hagihara et al, 1991) and suppress the development of atherosclerosis in the aorta of rabbits by inhibiting recruitment and proliferation of macrophages and formation of foam cells in plaques (Asai et al, 1993).

1.3 Non steroid hormones

1.3.1 Thyroxin

The hormones synthesized by the follicular cells of the thyroid gland were amongst the first observed to affect cholesterol metabolism. Hypercholesterolemia was a useful marker for the diagnosis of hypothyroidism in patients before the general availability of rapid measurements of thyroid function (Mason et al, 1930). Both the thyroid hormones, l-thyroxin (T4) and l-triiodothyronine (T3) are believed to have a variety of influences on the concentration of cholesterol. For example, in the presence of thyroid hormones, hepatic synthesis of cholesterol is actually enhanced (Rossner and Rosenqvist, 1974), adding to the concentration of total cholesterol, but these hormones also increase the fractional clearance rates of VLDL and LDL particles (Rossner and Rosenqvist, 1974) and the hepatic excretion of cholesterol (Miettinen, 1968). The net influence of the opposing actions of the hormones in the liver gives rise to greater concentrations of cholesterol in hypothyroid individuals compared with those with euthyroid or hyperthyroid levels of the hormones. In patients with hyperthyroidism, the concentration of HDL cholesterol and the ratio of LDL/HDL cholesterol are generally lower (Scottolini et al, 1980) than those in patients with hypothyroidism (Scottolini et al, 1980). *In vivo* studies in rodents show that thyroid hormone increases the concentrations of apoA-I protein and the corresponding mRNA (Mooradian et al, 1996), the same result was found in human liver tissue culture (Vandenbrouck et al, 1995). A Motif within the apoA-I promoter that resemble T3-response elements (TRE) (Taylor et al, 1996) is located between -208 and -193 in the rat apoA-I promoter, and corresponds to a similar

sequence in the human gene between –214 and -192 (Rottman et al, 1991). In hyperthyroid state, fatty acids oxidation and ketogenesis are stimulated simultaneously with paradoxical stimulation of fatty acid synthesis which may be due to stimulatory response of palmitoyltransferase I (CPT-I) to malonyl –CoA (Heimberg et al, 1985).

1.3.2 Leptin

The leptin (ob) gene was isolated by a group of molecular biologists at the Rockefeller University in December 1994 and its sequence patented. The protein coded of the ob gene was given the name leptin from the Greek word for thin, leptos. In 1995 several groups showed that leptin injections were capable not only of inducing dramatic weight reductions in very fat ob mice but were also able to reduce overfed normal mice.

Human OB is a 16 kDa, 146 amino acid (aa) residue non-glycosylated polypeptide and contains two cysteines in the carboxyterminal region, both of which are believed to participate in an intramolecular disulfide linkage (Leroy et al, 1996). Leptin hormone is primarily produced by adipose tissue (Laharrague et al, 1998) and other tissues such as bone marrow (Laharrague et al, 1998), placenta (Hoggard et al, 1997) and may be liver (Friedman-Einat et al, 1999).

In mouse, there is a notable mutation that occurs in the coding sequence number 106 (normally an arginine residue), here, a cytosine to thymidine change creates a stop codon that causes premature termination of the OB molecule. This mutation disrupts functional OB production, is associated with the ob-/ob- mouse and is accounted for select obese conditions in mouse but this situation does not appear to exist in humans (Maffei et al, 1996). The receptor for OB has been identified in mouse (Tartaglia et al, 1995), human (Lee et al, 1996) and rat (Lida et al, 1996).

In human, the mature receptor is 1142 aa residue and is a transmembrane protein with a predicted molecular weight of 81 kDa. The molecule shows 817 aa residues in its extracellular segment, 23 aa residues in its transmembrane domain, and 302 aa residues in its cytoplasmic

tail (Tartaglia et al, 1995). There are at least six isoforms of the leptin receptor (Ob-R) (Lee et al, 1996). Mouse, human and rat OB receptors are all virtually identical in length. Anatomical regions with soluble OB receptor expressed in adipose tissue stores, hypothalamus, cardio-vascular system, testis, cerebral cortex, cerebellum, choroid plexus, lung, kidney, skeletal muscle, liver, pancreas and adrenal medulla (Golden et al, 1997). Pancreatic β-cells that produce insulin have also been identified as expressing OB-Rs but unknown form (Kieffer et al, 1996). Human obesity is often associated with increased blood OB levels suggesting insensitivity to OB (Rohner-Jeanrenaud and Jeanrenaud, 1996). Insulin is suggested to be part of a negative feedback loop by stimulating OB secretion and the circulating OB inhibits insulin production (independently of feeding) (Mizuno et al, 1996). Insulin and the size of white adipocytes determine the actual quantity of OB released (Klein et al, 1996).

Increased OB (due to abundant fat stores) decreases neuropeptide Y (NPY) expression in the ventromedial hypothalamus, an action that activates the sympathetic nervous system and stimulates the heat producing activity of brown adipose tissue (BAT) resulting in increased whole body energy expenditure and weight loss (Tomaszuk et al, 1996), also NPY is a strong stimulator of appetite (Ahima et al, 1996).

1.3.3 Insulin and glucagon

Insulin is one of the most important hormones that control plasma glucose in a narrow range between 4 and 7 mM in normal individuals. Normal fasting serum insulin level was 10-400 μIU/ml (40.1± 23.4 nmol) (Matsui et al, 1998). It increases glucose uptake in muscle, liver and fat. It also stimulates cell growth and differentiation and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and inhibiting lipolysis, glycogenolysis and protein breakdown. Insulin receptor belongs to a subfamily of receptor tyrosine kinase that includes the insulin-like growth factor (IGF)-I and insulin receptor-related receptor (IRR). These receptors are tetrameric proteins consisting of two α and two β subunits that function as allosteric enzymes in which the α-subunit inhibits

the tyrosin kinase activity of the β-subunit. Insulin binds with α-subunit leads to increase kinase activity in the β subunit (Patti and Kahn, 1998). At least eight intracellular insulin receptor substrates (IRS) are activated by insulin, four are enumerated from 1-4 and other four are Gab-1, p60, CbI and APS (Saltiel and Kahn, 2001). IRS-1 has related to insulin growth promotion and insulin sensitivity in peripheral tissues, IRS-2 has insulin growth promotion in certain region (brain, retina and islet) and responsible for insulin sensitivity in both peripheral tissues and liver and IRS-3 and IRS-4 had slightly growth promotion (Saltiel and Kahn, 2001). PI(3)K has a role in metabolic (including glucose transport, glycogen and lipid synthesis) and mitogenic action of insulin (Shephered et al, 1995). Insulin also stimulates the mitogen-activated protein (MAP) kinase extracellular signal related kinase (ERK). This pathway involves the tyrosine phosphorylation of IRS protein and/or Shc, which in turn interact with adapter protein Grb2, recruiting the Son-of-sevenless (SOS) exchange protein to the plasma membrane for activation of Ras. Once Ras activated, it operates as a molecular switch and stimulates a serious kinase cascade through the stepwise activation of Raf**,** MEK and ERK. Activated ERK can translocate into nucleus where it catalyses the phosphorylation of transcription factors leads to cellular proliferation and differentiation (Boulton et al, 1991). Blockade of this pathway with dominant negative mutants or pharmacological inhibitors prevents the stimulation of cell growth by insulin, but has no influence on the metabolic actions of insulin (Lazar et al, 1995)**.** Insulin deficiency results in profound dysregulation of these processes and produces elevations in fasting and postprandial glucose and lipid levels.

In patients with type 1 diabetes, HDL-cholesterol decreases with increasing blood levels of glycated haemoglobin and increasing albuminuria (Laffel, 1999) and also associated with hyperacylemia and ketosis. Low HDL-cholesterol in these patients which results from decreasing of hepatic apoA-1 gene expression is the most frequent dyslipidemia in patient with type 1 diabetes increasing the cardiovascular risk (perez et al, 2000). Ketosis in type 1 diabetes which is due to accumulation of aceton, acetoacetate and ß-hydroxybutyrate, is

highly affecting macrophages and liver cells because of low activity of the rate-limiting enzyme in ketolysis i.e., succinyl-CoA-oxoacid transferase (Laffel, 1999). Acetoacetate downregulates ABCA1 gene expression especially in macrophages (Uehara, 2002). Hyperacylemia especially unsaturated fatty acids in type 1 diabetes can suppress ABCA1 expression in liver and macrophages of streptozotocin–induced diabetic mice (ie., a model for type 1 diabetes) (Uehara, 2002).

Glucagon is a single polypeptide chain, composed of 29 amino acids, and secreted from α-cells of islets of Langrehans. It contains no -S-S- bridges and needs no zinc for its crystallization. It is a hyperglycaemic hormone through stimulation of glycogenolysis and gluconeogenesis, protein catabolism and lipolytic activity through 3`5`cAMP. During stress, there is an excessive control of metabolism by the stress hormones including the corticosteroids, catecholamines, glucagon, and growth hormone. The actions of the stress hormones are generally opposed by insulin (Hardardottir et al, 1994). Stress hormones cause an increased breakdown of proteins, glycogen, and triglyceride to molecules that can be rapidly metabolized. Amino acids liberated from protein hydrolysis are utilized for the synthesis of glucose by gluconeogenesis. Glucose, from these sources, is utilized by the brain and is available as a source of energy in an acute stress situation. With repeated or chronic stress, stress hormones, together with fatty acids, cause insulin resistance in peripheral tissues, which may result in hyperglycemia (McEwen et al, 1997). Stress hormones elevate the level of homocysteine which induce a heightened state of cardiovascular activity, injured endothelium, and induction of adhesion molecules on endothelial cells to which recruited inflammatory cells adhere and translocate to the arterial wall (Stoney and West, 1997). glucagon from 0.01 to 5 ng/mL also induced a significant increase in rigidity index (RI), with the maximal impact being achieved using 5 ng/mL which could be involved in the pathogenesis of atherosclerosis (Valensi et al, 1993).

1.3.4 Luteinizing Hormone

The human luteinizing hormone (lutropin or LH) is a glycoprotein with a molecular weight of 30000, secreted by the basophilic cells in adenohypophysis. Like other glycoprotein hormones (FSH, TSH and HCG), LH contains two different subunits, an ∝- and a ß-chain, linked by noncovalent bounds. The primary structures of the ∝-subunits of LH and of those mentioned are virtually identical, whilst their ß subunits are different. The ß subunits are responsible for the immunological and biological specificity of these hormones (Nansel et al, 1979). The LH synthesis and release are stimulated by the hypothalamic gonadotropin releasing hormone (GnRH), whereas the ovarian steroids secreted from the corpus luteum control further secretions of LH by negative feedback. Luteinizing hormone affects key biochemical changes critical to normal menstrual and ovulatory function in reproductive aged women. LH is released by the anterior pituitary in hourly pulses as circhoral oscillations (Warner et al, 1983). This release is in response to pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the arcuate nucleus of the hypothalamus. GnRH was first isolated in 1970 and has a serum half-life of 20 minutes. Both estrogen and progesterone play important roles in modulating the release of LH, estrogen is more significant in early follicular development and ovulation. Estrogen, specifically estradiol, normally inhibits LH secretion. However, when estrogen reaches a certain level, there is positive feedback to the anterior pituitary, resulting in an increase in circulating LH (Hill et al, 1980). This transition from suppression to stimulation of LH secretion takes place at the mid–follicular phase and is dependent on the absolute level and duration of elevation of serum estradiol. Estradiol levels must be greater than 200 pg/ml for 50 hours to stimulate LH secretion (Hill et al, 1980). This typically occurs when the dominant ovarian follicle reaches a diameter of 15 mm or greater. The direct influence of the mid–follicular estradiol peak is the LH peak. LH is critical to luteinization of the ovarian follicle, production of progesterone in the theca cells, and the postovulatory follicular function. If estrogen levels are not sustained at these levels, the mid

cycle LH surge may be abbreviated or even fail to occur (Hill et al, 1980). Lewis and Wexler found that depression of circulating LH levels parallels the severity of the arteriosclerosis in rat (Lewis and Wexler, 1975).

1.3.5 Somatostatin

Somatostatin (SRIF) is a peptide hormone has two forms SRIF-14 and SRIF-28 reflecting their amino acids chain. Each isoform are secreted depend upon the tissue, for example, SRIF-14 is the predominant form produced in the nervous system and pancreas, whereas the intestine secretes SRIF-28 (Praydayrol et al, 1980). In addition, the two forms have different biological potencies for example SRIF-28 is ten fold more potent in inhibition of GH secretion, but less potent than SRIF-14 in inhibiting glucagon release (Mandarino et al, 1981). Because SRIF-14 and SRIF-28 inhibit glucagon and insulin release with different potencies from pancreatic α- and ß-cells, it has been postulated that each cell type expresses different SRIF receptor subtypes (Amherdt et al, 1989). Somatostatin is present in the hypothalamus, cerebral cortex, brain stem, gastro-intestinal tract, and pancreas. In the CNS, it acts as a neurotransmitter; its hormonal activities include inhibition of the release of growth hormone, insulin, glucagon, gastrin, TSH, ACTH, secretin, pancreozymin, cholecystokinin, pepsin and renin (Brazeau et al, 1973). SRIF and its receptors play an important role in the detection and therapy of neuroendocrine disorders including GH-secreting pituitary adenomas, and gastro-entero-pancreatic carcinoid tumour, vasoactive intestinal peptidomas, gastrinomas, insulinomas, and glucagonomas (Fehmann et al, 2000).

Abnormalities in plasma lipid profiles have been reported in patients with acromegaly and these changes may partly contribute to the increased cardiovascular risk of these patients (Bengtsson et al, 1988). In acromegalic patients, an increase in the concentrations of small dense LDL (Tan et al, 1999a) and remnant-like lipoprotein particles (RLP; Twickler et al, 2001) had been demonstrated. These patients with predominantly small dense LDL particles (Campos et al, 1992a) and exaggerated postprandial lipaemia with accumulation of

triglyceride-rich lipoprotein remnants (Masuoka et al, 2000) have an increased risk of coronary heart disease (CHD). Small dense LDL are more prone to oxidation in vitro (Tribble et al, 1992) and show an increased propensity to bind to arterial wall proteoglycans (Anber et al, 1996), whereas remnant-like lipoproteins are taken up by macrophages and can directly cause foam cell formation (Tomono et al, 1994). Both remnant-like lipoproteins and small dense LDL are also associated with endothelial dysfunction (Tan et al, 1999a).

Some of the changes in plasma lipids can be corrected after treatment of acromegaly with Octreotide, a somatostatin analogue, which is given as monthly intramuscular injections (Lancranjan et al, 1996). Serum cholesterol remained unchanged but serum triglyceride decreased and apoA-I increased after treatment (Oscarsson et al, 1994). A decrease in triglyceride (James et al, 1991), a rise in HDL cholesterol (Lam et al, 1993) and lower in the elevated apolipoprotein (a) levels (Lam et al, 1993) were also observed after octreotide treatment. But there are no reported data of the lowering of GH on small dense LDL and RLP in patients with acromegaly.

2. Aims of Work

Coronary artery disease (CAD) is one of the most common causes of death in the world. Not only dyslipoproteinemia but also reverse cholesterol transport may play a pivotal role in the maintenance of cellular lipid homeostasis and contribute to acceleration of atherosclerosis. Protection from pathological lipid accumulation in macrophages that reside in the vessel wall needs the understanding of the mechanisms involved in the regulation of genes involved in reverse cholesterol transport.

Members of the ABC transporter gene family such as ABCA1, ABCA7, ABCG1, ABCG5 and ABCG8 are major regulators of plasma HDL-cholesterol pool size as evident from identified mutations in the ABCA1 gene that causes familial HDL deficiency syndromes and mutations in the ABCG5 and ABCG8 genes leading to β-Sitosterolemia.

The first aim of my study was to perform an initial screening of hormones with known influences on plasma HDL to examine their effect on ApoA-1 dependent cholesterol and phospholipid effluxes. Next, a correlation of these effects with their influences on the gene expression of sterol-sensitive ATP-binding cassette transporters that are involved in the cellular efflux of lipids in human macrophages and HepG2 cells should be performed. Subsequent experminents with one hormone shown to strongly upregulate ABCA1 in order to elucidate the underlying mechanisms of gene expression on its receptor, intracellular pathway, transcription factors and post-transcriptional modifications will be carried out.

3. Materials and Methods

3.1 Cell culture

Human hepatoblastoma derived cells ATCC (HepG2) were obtained from (American Type Culture Collection). HepG2 cells were grown in Dulbecco´s modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (Sigma, St. Louis, USA) and incubated in 10% Co_2 in air at 37 °C. The cells were seeded into 6 cm or 10 cm dishes and grown to 70% confluence in complete culture medium. The medium was then removed and the monolayers were washed three times with phosphate buffered saline (PBS) before RNA isolation.

Human monocytes obtained from healthy donors were isolated by leukapharesis and counterflow elutriation (Klucken, et al 2000). Aliquots of 10 million cells were suspended with Macrophage-SFM medium, cultured in plastic Petri dishes (Gibco-BRL, Karlsruhe) and incubated overnight. After incubation, non adherent cells were removed by washing with warm Macrophage-SFM medium and incubated for additional 4 days with Macrophage-SFM medium in the prescence of 50ng/ml recombinant human macrophage–colony stimulating factor (M-CSF) (R&D system, Wiesbaden, Germany) to induce macrophages differentiation befor hormonal stimulation.

3.2 RNA isolation.

Total RNA was prepared by subsequent extraction and precipitation with Trizol (1ml Trizol to each 10^6 cells) (Sigma) (GibcoBRL/Life Technologies, Breda, the Netherlands) and precipation by centrifugation 15000 rpm for 2min at 25 C° in QIAshredder tube (QIAGEN). Chloroform was added and mixed. The suspension was centrifuged at 17000 rpm for 15 min. A second phenol/chloroform extraction was performed by centrifugation 17000 rpm for 10 min, followed by an ethanol (75%) precipitation. The air dried pellet was dissolved in 100 µl RNase free water and further purified with a RNeasy mini column (Qiagen GmbH,Hilden, Germany) (Baelde, et al 2001). Total RNA was resuspended in RNase free water (Roche) and

its quantity and quality were determined by ultraviolet spectroscopy and electrophoretic analysis on an Agilent 2100 Bioanalyzier with RNA 6000 LabChip. Only total RNA with an absorbance ratio (*A*260 /*A*280) more than 1.8 and a ribosomal RNA (28S/18S) ratio of 1.8-2.2 was used. The intergrity and size distribution of total RNA can be checked by denaturing agarose gel electrophoresis (1.2%) and ethidium bromide staining. The respective ribosomal bands should appear as sharp bands on the stained gel. The 28S ribosomal RNA band (human: 5.0 kb) should be present at approximately twice the amounts of the 18S (human: 1.9 kb) RNA.

3.3 Reverse transcription

Using the 1st strand cDNA synthesis kit (AMV) from Roche (Basel, Switzerland), RNA was transcribed into single strand cDNA using randam hexamer primers. The reaction was composed of 2µL of 10X reaction buffer ,4 µL 25 mM Mgcl2 (5mM), 2 µL deoxyribo-nucleotides mix (1mM), 2µL random hexamer primers, 1µL RNase inhibitor (50 units), 0,8 µL AMV-Reverse Transcriptase (20 units) and 1 µg RNA in a total volume of 20 µL. The mix was briefly vortexed, centrifuged and incubated at 25 °C for 10 minutes (primer annealing), 42 °C for 60 minutes (Reverse Transcription) and 95 °C for 5 minutes (Reverse Transcriptase deactivation).

3.4 Relative quantification by TaqMan™ real time RT-PCR.

Real time PCR, using the ABI Prism HT7900 (PE Applied Biosystems, Foster city California, USA.) was performed in a total volume of 20 µL in a 384 wells plate. The reactions contained a Taqman master mix 18 µM forward primer, 18 uM reverse primer, 5 µM probe and 50 µg cDNA for each reaction. The relative quantities of target genes were normalized with 18S-ribosomal RNA (18SrRNA) to compensate for variation in input cDNA. The primers and probes were designed using primer express 2.0 SDS software based on sequence entries in the Gene bank. The designed primers and probes are listed in table (1). The TaqMan probes were labelled with a reporter dye (FAM) on its 5`end and a quencher dye (TAMRA) on its 3`end.

The probe for 18S RNA was labelled with a reporter dye (VIC) on its 5`end and TAMRA on its 3`end. Measurements were carried out in triplicate and quantification was performed relative to standard curve for 18SrRNA. The results were analysed with an ABI sequence detector software version 2.0 (PE Applied Biosystems).

	Primer Forward 5`→3`	**Primer Reverse 5`→3`**	**Probe 5`→3`**
ABCA1	tgt cca gtc cag taa tgg tc tgt	cga gat atg gtc cgg att gc	tac acc tgg aga gaa gct ttc aac gag act aac c
ABCA2	aag cct gtg gag gat gat gtg	ggt caa cgg cca gga tac g	tgg cca gtg agc ggc agc g
ABCA3	caa aac cct gga tca cgt gtt	cct ccg cgt ctc gta gtt ct	tgc tgc cca acc act gtc tgg g
ABCA4	aaa ggt tgc aaa ctg gag tat taa gag	ttg ttg ccc cca ctg tac gt	ctg ggc ctg act gtc tac gcc ga
ABCA5	ggc tgc tat tct gac cac tca cta ta	tta act gcc cag aca cca tga t	cag agg ctg tct gtg atc gag tag c
ABCA6	cca tga gaa atg tcc agt ttc ct	tgc tgg gtt aaa tta gat att ggt gta	tcc tca gaa tct ggg aag ggt aga taa a
ABCA7	ttt ctc tgg gac atg tgt aac tac ttg	tgt gat cga cca gcc ata cag	cct tcc agc aga ggg cat atg tg
ABCA8	tgc tta gtc cct ttg cct tca	gag ccg tcc gat gga tga	aat ggc cca gct ttt aca ctt gga cta tga tt
ABCA9	cat tca gac tga cag aag cac att t	tca cca atg ctg ctc att gc	tgg att atg agt atg ggt acc gaa gta a
ABCA10	atc aca aac tgc gtt tct cct tt	cag agg cct gaa atc cat aac tg	atc gcc atg agcagc atc agc ga
ABCA12	cct cca ttc agc acc aaa gtc t	tga ggt cac cca tgc cat t	cct acc tgt cac tcc tac ggg cac tcg
ABCB1	cag aca gca gga aat gaa gtt gaa	tga aga cat ttc caa ggc atc a	ttt cac ttt tgg att cat cag ctg cat ttt cta
ABCB2	ggg tga cgg gat cta taa caa ca	cca aac acc tct ccc tgc aa	cat ggg cca cgt gca cag cc
ABCB3	gag gag cat gaa gtc tgt cgc tat	ggc gcg ttc cag gtc tct	agg ccc ttg aac aat gtc ggc agc
ABCB4	tca gga agc cag atc cag tca	cga gat ttc cag cca ttt gg	agt ggc agc ctt ttc atc att tag ttc aaa ttc tt
ABCB5	gac atc ggc tgg ttt gat agc t	gag ttt cca gcc ctt cac ca	tga cat cgg tga act taa cac tcg act
ABCB6	tga aag agg aga cag aag tga agg a	aac tca ata cgg ccc ttc tga a	ctg gag cag ggc ccc ttc gc
ABCB7	gat ccg gcc ttt agt ctc tgt tag	cca agg cgc cga gtt g	agg tcc gcagtg gag gcc aca
ABCB8	gaa gcg aat gct cac gag ttc	tca ccg acg acc gtg ttg	tca cca gct tcc ccg agg gct
ABCB9	cgc cca cct cca acc a	cgc aga tgt cca cac tca tga	agg atg cgg ctg tgg aag gcg
ABCB10	gct tcc gta ggc atc agt atg at	gga ggc acc acg ctc aaa	ttt ttt gtc tca cct aat ctg gcc acc ttt g
ABCB11	agg gag cta cca gga tag ttt aag g	tcg tgc acc agg taa gaa agc	ctt cca tcc ggc aac gct cca
ABCC1	gaa ggc cat cgg act ctt ca	cag cgc gga cac atg gt	ctc ctt cct cag cat ctt cct ttt cat gtg
ABCC2	tgc agc ctc cat aac cat gag	gat gcc tgc cat tgg acc ta	aga gag aac agc ttt cgt cga aca ctt agc c
ABCC3	cac acg gat ctg aca gac aat ga	gag ttt cca gcc ctt cac ca	cca gtc acc tat gtg gtc cag aag cag ttt
ABCC4	aag tga aca acc tcc agt tcc ag	ggc tct cca gag cac cat ct	caa acc gaa gac tct gag aag gta cga ttc ct
ABCC5	tga aag cca ttc gag gag ttg	tga aag cca ttc gag gag ttg	ctc gca gcg tgc cct tga caaa ag
ABCC6	aga cac ggt tga cgt gga cat	gct gac ctc cag gag tcc aa	cca gac aaa ctc cgg tcc ctg ctg at
ABCC7	ggc acg aag gag gca gtc	tcg tgt gga tgc tgt tgt ctt t	tga tga cac act cag tta acc aag gtc a
ABCC8	cac caa tca gct cat gtg gtt t	ggc act gac tcc gag tat gta gta ga	cca gta cag atc att gtg ggt gtg at
ABCC9	cta ctc ctg tgt tcc tgg ttg ct	caa ccc gaa agt att tct gga taa a	tcc tgc ccc ttg gtg ttg c
ABCC10	gcg ggt taa gct tgt gac aga	ccc accc cgc aga act tga	ctg ctg agt ggc att cgg gtc
ABCC11	agg gtc tac cac cac tac atc ca	cga tca gca cca cga aga ag	cag ctg gag gtt aca tgg tct ctt gca taa tt
ABCC12	ttc atc caa agg cct gtc att	ccg ttc gca cac aca ctt g	cat aca tca tcc agc tga gcg gac tgc t
ABCD1	cct ctt tct aca gcc taa ttt att gga	tgg cac ggt agt cac att gg	tcc cta ttc gta gcc atc tcc g
ABCD2	gct acc ttc gtc aac agt gca a	cgt ggt cta ggc gag ttc tg	agg tac ctg gaa tgc aaa ttg gct
ABCD3	acc cct ctc agt ctg cag tat tg	tga tac atg gta acc cct cct tgt	tgt tta aag tat atg tgc agt ctt gct
ABCD4	ggc cca ggt tag atc tgc aa	tga tga cca aga agg aaa caa aac	cca gcg gtt cct gca gat act ga
ABCE1	gat cgc gtc atc gtt ttt ga	atg cca gcc aaa agg gtt t	tgt tcc atc taa gaa cac agt tgc aaa c
ABCF1	gaa gtt cag cat ctc cgc tca t	ggc ggc cgg cta caa	agg agc tgt tcg tca atg cag acc tgt ac
ABCF2	tgg agc agg gaa gtc aac tct t	ttt tcg gat cat gcc atc tg	tga agc tgc taa ctg gag agc tac tac c
ABCF3	ccg gga gtt gtg ggt atg c	gta ctg gtc aaa tcc tcc ttc ca	aag gag gcg gcg tca ccc g
ABCG1	ccg acc gac gac aca gag a	gca cga gac acc cac aaa cc	tct gat cca acc cct aga acc ggg t
ABCG2	cag gtc tgt tgg tca atc tca ca	tcc ata tcg tgg aat gct gaa g	cca ttg cat ctt ggc tgt cat ggc tt
ABCG4	gag cca ggg tca gtg cat ct	gca agc cga gtc ctt tta ga	caa agg cgt ggt cac caa cct gat c
ABCG5	tct ctt ggc ccc cca ctt a	cta tat ttg gat ttt gga cga tac ca	ttg gtg aat ttc taa ctc ttg tgc t
ABCG8	tcg tac cct ctc tac gcc atc t	ggc cag gta cag gac cat gaa	ggt cat tgg cct cag cgg t

Contineous table (1)

PPARα	gaa atg gga aac atc caa gag att	ggg ctc gaa gct ggt gaa	tca tca cgg aca cgc t
PPARγ	agg cga ggg cga tct tg	ccc atc att aag gaa ttc atg tca	gca gga aag aca aca gac aaa tca cca ttc gtt
PPARδ	cat cct cac cgg caa agc	atg tct cga tgt cgt gga tca c	aca cgg cgc cct t
SDP1	aga gga agg ccg aga gag atg	tca ggt gcc tat ggt tcc a	tgg cgg gag tca tga tat ttt cca cc
KAP1	ata ggc agc cat aat tca gaa act c	tct gac agc agg cca tgg t	tcc aag caa ccc aac ctt cag atc aac t
ZNF202	aga aga ggg aat tct gat ggt gaa	ccg ggt cat ccc tct gta ag	aga tga ttt cac ctg tcg gcc aga gtc tg
ZNF195	tcc tgg gct caa gcg atc t	cac ggt ggt tca cac ctg taa t	cct gcc tca gcc tcc caa agt gc
LXR-α	tgc ccc atg gac acc tac at	cca gcc tga cgg cat ttg	cgc aag tgc cag gag tgt cgg c
ER-α	ggg cca ctg ggt tgg aa	gta aga ttt caa gag gta ttc ata gaa gga	cag agt ggc ctg ggt gcc gg
ER-β	gcc gac aag gag ttg gta cac	cag gct gag ctc cac aaa gc	tga tca gct ggg cca aga aga ttc cc

Table (1):- ABC gene subfamily, PPARs, SDP1, KAP1, ZNF202, ZNF195, LXR-α and ERs primers (forward and reverse) and probes. Both are designed by using primer express 2,0 SDS software (ABI) based on the sequence entries in the Gene bank. Both primers and probe wrot from 5` (left) to 3` (right). The probes of ABC subfamily were labelled with a reporter dye (FAM) on 5`end and the quencher dye (TAMRA) on 3`end.

3.5 Cholesterol and phospholipid effluxes

Assays for ^{14}C-cholesterol and ^{3}H-choline-phospholipid effluxes were performed with confluent HepG2 and macrophage cells on 6 wells dishes as described by Klucken and coworkers (2000) (Klucken et al, 2000). One million HepG2 cells were maintained in DMEM containing 10% FCS and 1.5 μCi/ml 14 C-cholesterol (Amersham, Freiburg, Germany) for 3 days. Cells were than rinsed with phosphate buffered saline (PBS) and incubated in DMEM containing 5 mg/ml fatty acid free bovine serum albumin (BSA) with 30 μg of cholesterol for 24 h, followed by 36 h in the same culture medium without cholesterol to allow equilibration of cellular cholesterol pools in loaded cells. Choline phospholipids were labelled by adding 10μCi/ml ^{3}H-choline chloride (Amersham, Freiburg, Germany) to the culture medium for the final 24 h of the equilibration incubation. Cells were then rinsed with PBS and incubated for 18 h in DMEM containing 2 mg/ml BSA with or without 10 μg/ml apoA-I and the indicated hormone.

Monocyte-derived macrophages were cholesterol loaded by adding M-CSF-containing serum-free macrophage medium supplemented with 40mg/ml enzymatically modified LDL (E-LDL) and 1.5 μCi/ml ^{14}C-cholesterol as well as 10 μCi/ml ^{3}H-choline chloride. After 24 h incubation, cells were rinsed with PBS and incubated for 18 in RPMI medium supplemented with L-glutamine and with or without 10 mg/ml apoA-I and the indicated hormone. The final

ethanol concentration in medium was less than 0.1%. Culture media were harvested and centrifuged for 10 min at 10.000 rpm to eliminate remaining cell debris. The cell was washed with PBS and lysed with 0.2% SDS. The effluxed cholesterol and choline was precipitated with chloroform and the radioactivity of ^{3}H- and ^{14} C- in the cells and culture medium was determined by liquid scintillation counter as percent fraction of the cpm. Basal effluxes were calculated on the difference between ^{3}H-choline-phospholipid or ^{14}C-cholesterol of the media and that of the intracellular without adding apoA-I. The total effluxes were the difference between ^{3}H-choline-phospholipid or ^{14}C-cholesterol of the media and intracellular site after adding apoA-I. Specific apoA-I dependent effluxes of ^{3}H-choline-phospholipid or ^{14}C-cholesterol were the difference between both the total effluxes and basal effluxes. Specific apoA-I dependent effluxes of ^{3}H-choline-phospholipid or ^{14}C-cholesterol in HepG2 cell line or human macrophage were considered as 100% and compared by that after incubation with hormonal incubation. Three independent experiments with triplicate measurements have been performed in both HepG2 cells and human macrophages.

3.6 Western Blot

20 μg postnuclear lysates were loaded into a 7.5% Tris-HCL SDS gels (Bio-RAD) in sample buffer without boiling. Following electrophoresis, the gel was transferred to a nitrocellulose membrane at 30 volt overnight. Blocking of the membrane was performed with 5 % dry milk in TBS-T (Tris-buffered-saline with 0.1% tween 20) for 1 h. The membrane was washed with TBS-T for 5 min, probed with anti-ABCA1 polyclonal antibody 1:500 (from Novus Biologicals) for 1 h at room temperature. The membrane was washed with TBS-T three times for 10 min each, incubated with donkey antirabbit secondry antibody (Amersham) at 1:2000 in 3% milk in TBS-T for 1 h and washed with TBS-T 3 times, 10 min each. After final washing with TBS-T, a chemiluminescent reagent (Pierce) was added to the membrane and finally exposed to X-ray film for one minute (Drobnik et al, 2002).

3.7 Data analysis

The Sequence Detector Software SDS 2.0 (Applied Biosystems) was used for data analysis. The first step was to generate an amplification plot for every sample, which showed ΔRn on the *y* axis (where Rn is the fluorescence emission intensity of the reporter dye normalized to a passive reference, and ΔRn is the Rn of an unreacted sample minus the Rn value of the reaction) against the cycle number, displayed on the *x* axis. From each amplification plot, a threshold cycle (Ct) value was calculated, which is defined as the cycle at which a statistically significant increase in ΔRn is first detected and is displayed in the graph as the intercept point of the amplification curve with the threshold. The threshold is automatically calculated by SDS as the 10-fold SD of Rn in the first 15 cycles. The obtained Ct values were then exported to a Microsoft Excel spreadsheet for further analysis.

The next step was to construct calibration curve plots, using Microsoft Excel as recommended in User Bulletin 2 for the ABI Prism 7700 Sequence Detection System (Applied Biosystems), showing Ct values on the *y* axis and the logarithm of the input amount of cDNA (equivalent to the amount of total RNA) on the *x* axis.

All human ABC transporters were subsequently measured in the different human tissues, and the obtained Ct values were used to calculate the initial input amount. Thereafter the results were normalized to the endogenous control, 18 S-ribosomol RNA (18SrRNA).

In the last step, we compared the expression of each individual ABC transporter in the complete tissue panel. Therefore, for each ABC transporter, the normalized amount of expression in the tissue that showed the lowest expression was used as a calibrator (set to 1), and the remaining tissue samples were displayed as fold changes (Langmann et al, 2003).

4. Results

4.1 Cholesterol and choline-phospholipid effluxes in human macrophages and HepG2 cells.

In order to analyse cellular effluxes of cholesterol and choline-phospholipid effluxes, cells were pulsed with ^{14}C-cholesterol and ^{3}H-choline-phospholipid and chased with apoA-I for specific efflux. *In vitro* differentiated human macrophages, one steroid or non-steroid hormone was added.

Estrogen (100 nM for 36h) increased specific apoA-I dependent cholesterol and choline phospholipid effluxes (235% ± 6.4% $p<0.05$ and 185% ± 6.6% $p<0.05$ respectively), Progesterone (1 µM for 60h) had the same but less extend influence on specific apoA-I dependent cholesterol and choline phospholipid effluxes (193% ± 8.8% $p<0.05$ and 155% ± 15.9% $p<0.05$ respectively). Hydrocortisone (1 µg/ml media for 24 h) significantly inhibited specific apoA-I dependent cholesterol efflux (74% ± 6%) (tab. 2-A and B) and (Fig. 4A).

In contrast, some of Non-steroid hormones as Thyroxin (T3; 10 nM for 24h) significantly inhibited specific apoA-I dependent cholesterol and choline phospholipid effluxes (67% ± 5.5% and 88% ± 2.2 respectively) and glucagon (5 nM for 8h) also significantly decreased specific apoA-I dependent cholesterol and choline phospholipid effluxes (44% ± 9.8% and 34% ± 20.3% respectively). Human insulin (150 nM for 18h) augmented specific apoA-I dependent cholesterol and choline-phospholipid effluxes (235% ± 10.2% $p<0.05$ and 194% ± 28.1% $p<0.05$ respectively) (tab.2-C and D) and (fig.4B).

Hormones	Basal and total Efflux Cholesterol Efflux												Specific ApoA-1 Cholesterol Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
Mac.	3.6%	0.2%	8.6%	0.7%	3%	0.1%	8%	0.5%	2.2%	0.2%	6.4%	1.1%	100%	100%	100%	100%	0%
Estrogen	3.6%	0.4%	15.1%	1%	1.5%	0.1%	13.7%	1.2%	1.1%	0%	11%	0.1%	229.8%	242.1%	233.2%	235%*	6.4%
Progesteron	2%	0.3%	11.2%	1.2%	2.5%	0.1%	12.2%	1.3%	2%	0.3%	10.5%	2%	184.5%	192.1%	202.1%	192.9%*	8.8%
Androgen	1.9%	0.3%	7.1%	1.1%	3%	0%	7.8%	0.5%	4.6%	0.7%	8.8%	2%	105.4%	95.4%	98.7%	99.8%	5.1%
Dexamethasone	1.6%	0.2%	7%	2.4%	2.5%	1.6%	7.6%	1.1%	2.1%	0.2%	6.1%	0.4%	109.8%	101.2%	93.3%	101.4%	8.3%
Hydrocortison	2.8%	0.2%	6.4%	1.4%	1.4%	1.4%	5.4%	1.7%	1.7%	0.1%	4.6%	0.3%	73.3%	79.7%	67.8%	73.6%*	6%

Table (2-A)

Hormones	Basal and total Efflux Phospholipid Efflux												Specific ApoA-1 Phospholipid Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
Mac.	3,66%	0.3%	6,93%	0.3%	3.1%	0.2%	4.5%	0.4%	5.4%	0.1%	9.2%	1%	100%	100%	100%	100%	0%
Estrogen	4,78%	0.4%	10,81	2%	1.8%	0.4%	4.4%	0.7%	1.3%	0.5%	8.1%	1.4%	184.3%	191.3%	178.1%	184.6%*	6.6%
Progesteron	2,93%	0.5%	8,52	0.7%	3.6%	0.9%	5.8%	0.4%	4.4%	0.3%	9.8%	0.4%	171%	153.3%	139.4%	154.6%*	15.9%
Androgen	0,52%	0.1%	3,69	0.7%	3.2%	0.7%	4.3%	0.7%	5.7%	0.8%	9.1%	0.9%	96.8%	80%	89.1%	88.6%	8.4%
Dexamethasone	1,06%	0.1%	4,36	0.7%	2.6%	1.7%	4.2%	0.3%	4.6%	0.6%	8.5%	0.8%	100.9%	112.9%	102.9%	105.6%	6.4%
Hydrocortison	4,82%	0.6%	8,19	0.8%	2.3%	2.5%	3.4%	0.7%	2.7%	0.2%	5.7%	0.2%	103.1%	79.4%	78.4%	87%	14%

Table (2-B)

Hormones	Basal and total Efflux Cholesterol Efflux												Specific ApoA-1 Cholesterol Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
Mac.	3.6%	0.6%	8.6%	0.7%	3%	0.1%	8%	0.5%	2.2%	0.2%	6.4%	1.1%	100%	100%	100%	100%	0%
LH.	3.9%	0.6%	8%	1.3%	2.8%	0.3%	7.8%	0.8%	1.4%	0.3%	6.2%	0.5%	81.1%	99.7%	112.5%	97.8%	15.8%
Somatostatin	7.3%	0.2%	12.1%	0.8%	3.2%	0.1%	9.7%	0.1%	2.1%	0.1%	5.4%	0.5%	95.8%	128.1%	77.6%	100.5%	25.6%
Thyroxin(T3)	7.8%	1.1%	10.9%	1.4%	2.8%	0.2%	6.1%	0%	2.8%	0.5%	5.9%	0.2%	62.5%	64.9%	72.9%	66.8%*	5.5%
Glucagon	1.8%	0.4%	7.1%	0.8%	1.5%	0%	4.9%	0%	1.6%	0.3%	3.4%	0.7%	53.5%	34%	44.2%	43.9%*	9.8%
Insulin	5.8%	0.5%	28.7%	0.3%	2.7%	0.4%	25.8%	0.1%	2.1%	0.2%	12.5%	1.7%	228.9%	228.9%	246.6%	234.8%*	10.2%
Leptin	3.7%	0.6%	10.3%	0.4%	2.8%	0%	8.4%	0%	3.2%	0.4%	7.2%	0.4%	133%	109.4%	95.1%	112.5%	19.1%

Table (2-C)

Hormones	Basal and total Efflux Phospholipid Efflux												Specific ApoA-1 Phospholipid Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
Mac.	3,66%	0.3%	6,93%	0.3%	3.1%	0.2%	4.5%	2.4%	5.4%	0.1%	9.2%	1%	100%	100%	100%	100%	0%
LH.	3,15%	0.2%	4,97%	0.9%	2.7%	0.2%	4.1%	0.4%	2.8%	0.2%	5.5%	1.2%	55.7%	95.2%	70.4%	73.8%	19.9%
Somatostatin	5,77%	0.3%	9,15%	0.4%	3.5%	1.1%	4.4%	0.3%	3.7%	1.2%	7.8%	0.3%	103.4%	63.6%	95.5%	87.5%	21.1%
Thyroxin(T3)	5,63%	0.6%	8,52%	1.2%	3.3%	0.4%	4.6%	1.5%	5.1%	1.1%	8.4%	0.4%	88.3%	90.3%	85.9%	88.2%*	2.2%
Glucagon	6,34%	0.5%	7,8%	0.1%	3.4%	0.1%	3.5%	1.5%	2%	0%	3.8%	0.4%	43.2%	10.3%	47.4%	33.6%*	20.3%
Insulin	4,67%	0.3%	12,1%	1.6%	5%	0.3%	7.6%	0.5%	8.1%	0.1%	14.7%	1.6%	225.7%	184.3%	172%	194%*	28.1%
Leptin	5,02%	1%	8,5%	1.1%	3.6%	0.5%	4.8%	0.6%	4.6%	1%	6.9%	0.2%	106.9%	87.1%	59.3%	84.4%	23.9%

Table (2-D)

Table (2):- Effect of steroid and nonsteroid hormones on ApoA-1 dependent lipid effluxes:-

Influences of steroid hormones on basal, total and specific ApoA-1 dependent cholesterol (3-A, C) and phospholipids effluxes (3-B, D) in human macrophage were enumerated after stimulation with steroid hormones (3-A, C) "β-estradiol (100 nM for 36 h), progesterone (1 uM for 60 h), androgen (1 uM for 60 h), dexamethasone (10^{-7}M for 16 h) and hydrocortisone (1ug/ml for 24 h)" and non steroid hormones(3-B,D) "LH (5 ug for 16 h), somatostatin (2 ug/ml for 18 h), thyroxin (10 nM for 24 h), glucagon (5 nM for 8 h), insulin (100 nM for 18 h) and leptin (500 ng for 24 h)". Data were expressed as mean value in %, SD from three independent experiments. Specific ApoA-1 cholesterol and phospholipids effluxes of each experiment (Exp.1-3) were expressed in %, mean of the three experiments ±SD. * symbol means significant ($P \leq 0.05$).

Fig.4-A

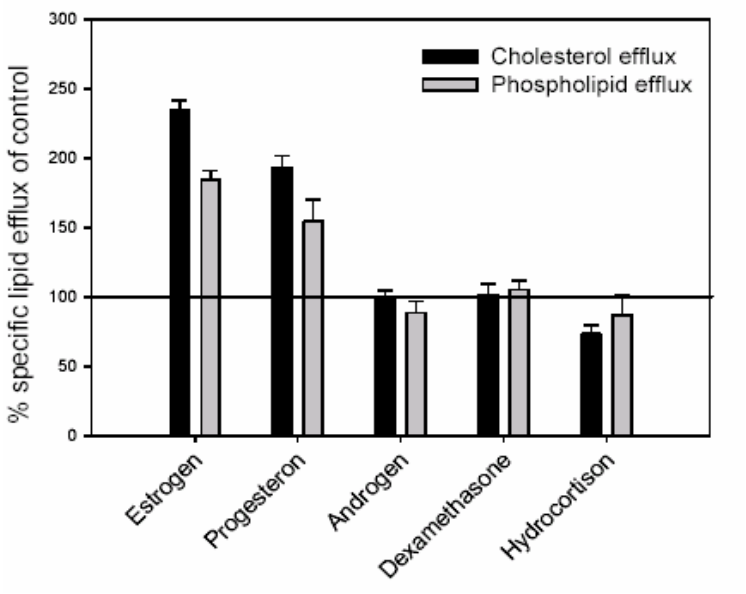

Fig.4-B

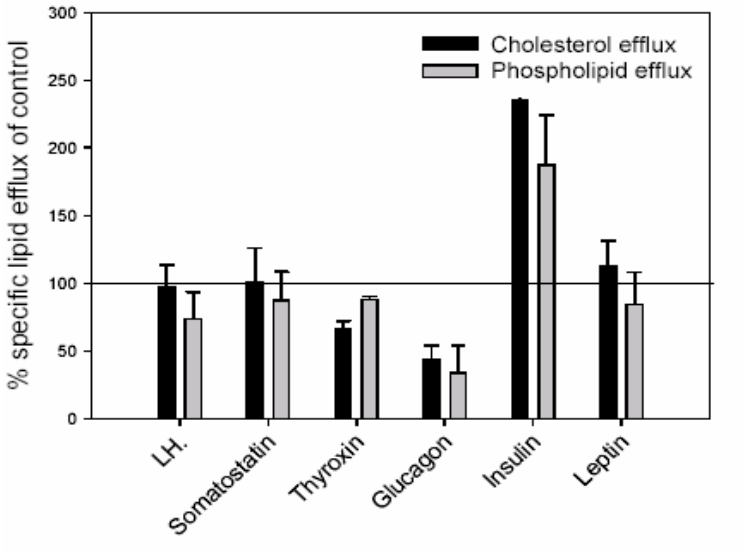

Fig.4. Effect of steroid and non steroid hormones on ApoA-1 dependent lipid effluxes in macrophages.
Influences of steroid hormones (fig.1-A) and non steroid hormones (fig.1-B) on specific ApoA-1 dependent cholesterol (black) and phospholpids effluxes (grey) in human macrophage were drawn in columns, vertical bars indicated SD, and the horizontal line passing through 100 % represented the specific ApoA-1 lipid effluxes without hormonal stimulation.

In HepG2 cells, 36 h incubation of β-estradiol (100 nM) in these cells decreased specific apoA-I dependent choline-phospholipid efflux (65% $\pm$ 5.2% $p<0.05$). Androgen (1uM for 60 h) could lower the specific apoA-I dependent cholesterol efflux to less extend (85% $\pm$ 6.2% $p<0.05$). Dexamethasone (10^{-7} M for 16 h) also supressed the specific apoA-I dependent cholesterol and choline-phospholipid effluxes (49% $\pm$ 7.3% $p<0.05$ and 42% $\pm$ 3.8% $p<0.05$ respectively) (tab.2 A-B) and (fig.5A).

18 h incubation of 150 nM human insulin in HepG2 cells increased the specific apoA-I dependent cholesterol and choline-phospholipid effluxes (274% $\pm$ 12.8% $p<0.05$ and 255% $\pm$ 17.6% $p<0.05$ respectively), whereas thyroxin (T3; 10 nM for 24 h) had the opposite influence (74% $\pm$ 5.4% $p<0.05$ and 57% $\pm$ 7.1 $p<0.05$ respectively). Both LH (5 ug/ml media for 16 h) and somatostatin (2 ug/ml media for 18 h) significantly increased specific apoA-I dependent choline-phospholipid effluxes to 211% $\pm$ 5.4% and 261% $\pm$ 31.4% respectively (tab.2 C-D) and (fig.5B).

Hormones	Basal and total Efflux Cholesterol Efflux												Specific ApoA-1 Cholesterol Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
HepG2	1.7%	0.4%	4.8%	0.5%	1.7%	0.3%	7.2%	1.3%	2.2%	0.1%	5.2%	1.1%	100%	100%	100%	100%	0%
Estrogen	1.8%	0.1%	5.3%	0.3%	1.5%	0.2%	8%	1.9%	3%	0.1%	7.4%	1.4%	109.6%	118.7%	120.2%	116.2%*	5.7%
Progesteron	2.3%	0.4%	5.7%	0.5%	1.5%	0.2%	7.4%	1%	2%	0.5%	5.7%	1.5%	107%	108.2%	100.3%	105.1%	4.3%
Androgen	1.5%	0.3%	4.2%	1.1%	1.2%	0.1%	5.6%	1%	1.9%	0.3%	5.3%	0.8%	84.3%	79.4%	91.7%	85.1%*	6.2%
Dexamethasone	1.9%	0.2%	3.6%	0.9%	2.1%	0.5%	4.3%	0.4%	2.3%	0.4%	4.2%	0.7%	54.5%	40.9%	52.1%	49.2%*	7.3%
Hydrocortison	3%	0.2%	6%	1%	2.8%	0.3%	8.2%	1.1%	3.1%	0.7%	6.3%	1.2%	95.2%	98.2%	87.1%	93.5%	5.7%

Table (3-A)

Hormones	Basal and total Efflux Phospholipid Efflux												Specific ApoA-1 Phospholipid Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
HepG2	1,68%	0.2%	3.4%	1%	5.4%	1.6%	13.3%	3.6%	4.1%	1.2%	9.1%	2.6%	100%	100%	100%	100%	0%
Estrogen	2.6%	0.1%	3.8%	0.3%	7.5%	2.1%	12.5%	3.7%	3.1%	1.1%	6.2%	2.2%	71.2%	63.8%	61.1%	65.4%*	5.2%
Progesteron	2.7%	0.4%	4.6%	0.9%	2.3%	0.9%	10.1%	2.4%	4.8%	1.5%	9.7%	2.3%	109.8%	97.5%	98.1%	101.8%	6.9%
Androgen	3.6%	0.5%	5.3%	1.1%	2.3%	1%	9.3%	3%	3.5%	1.6%	8.6%	3%	95.4%	88.4%	101.3%	95%	6.5%
Dexamethasone	2.7%	0.2%	3.4%	1.1%	7.3%	2.2%	10.3%	3.4%	3%	1%	5.2%	2.5%	43.9%	37.5%	44.3%	41.9%*	3.8%
Hydrocortison	2.3%	0.2%	4.1%	1%	2.8%	1%	12.1%	3.2%	3.4%	1%	9.4%	3.2%	99.3%	117.4%	120.1%	112.3%	11.3%

Table (3-B)

Hormones	Basal and total Efflux Cholesterol Efflux												Specific ApoA-1 Phospholipid Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
HepG2	1.6%	0.4%	4.8%	1.5%	1.7%	1.5%	7.2%	1.3%	2.2%	0.1%	5.2%	2.1%	100%	100%	100%	100%	0%
LH.	1.3%	0.3%	4.6%	1%	2%	0.2%	8.2%	2.1%	1.7%	0.2%	5.5%	1.3%	106.3%	114.7%	102.9%	108%	6.1%
Somatostatin	1.4%	0.2%	5.1%	1.1%	0.9%	0%	6.7%	2.5%	3.3%	1%	7.7%	2%	117.2%	105.2%	119.6%	114%	7.7%
Thyroxin(T3)	2.7%	0.5%	5.2%	0.2%	4%	1%	7.8%	2%	2.2%	0.1%	4.9%	0.5%	79.9%	69.3%	73.1%	74.1%*	5.4%
Glucagon	0.8%	0.1%	4%	1.2%	2.5%	0.5%	8.5%	1.6%	2%	0.4%	5.9%	1.2%	101.4%	109.9%	104.6%	105.3%	4.3%
Insulin	3.3%	0.3%	11.8%	3.2%	2.1%	0.4%	16.6%	3.5%	2.2%	0.4%	12.9%	3%	268.8%	265.1%	288.8%	274.2%*	12.8%
Leptin	0.8%	0.1%	4%	1.2%	2.7%	0.5%	8.6%	2%	3.2%	1%	6.4%	1%	101.3%	106.7%	88.1%	98.7%	9.6%

Table (3-C)

Hormones	Basal and total Efflux Phospholipid Efflux												Specific ApoA-1 Phospholipid Efflux				
	Exp.1				Exp.2				Exp.3				Exp.1	Exp.2	Exp.3	Mean	SD.
	Basal Efflux		Total Efflux		Basal Efflux		Total Efflux		Basal Efflux		Total Efflux						
	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.	Value	SD.					
HepG2	1,68%	0.5%	3.4%	1.0%	5.4%	1.6%	13.3%	3.6%	4.1%	1.2%	9.1%	2.6%	100%	100%	100%	100%	0%
LH.	5,89%	2.2%	10.6%	3%	3.7%	1.2%	20.2%	4.2%	3.7%	0%	14.1%	3.9%	217.2%	208.6%	207.3%	211%*	5.4%
Somatostatin	2,48%	1%	7.6%	2%	1.1%	0.5%	19.5%	3.4%	2.2%	0.2%	15%	3%	295.2%	233.1%	255.6%	261.3%*	31.4%
Thyroxin(T3)	2,45%	84%	3.3%	1.2%	5.6%	1.2%	10.3%	2.6%	2.8%	0.1%	5.9%	1%	49.1%	60.2%	62.3%	57.2%*	7.1%
Glucagon	6,67%	2.1%	8.7%	2.1%	5.8%	1.5%	15.7%	3.6%	4.1%	1.2%	8.4%	2.1%	115.1%	124.5%	85.2%	108.3%	20.5%
Insulin	1,92%	0.3%	6.6%	2%	2.1%	0.3%	20.7%	4.3%	1.7%	0%	14.8%	3.5%	269.6%	235.6%	260.9%	255.3%*	17.6%
Leptin	4,36%	2.1%	6.2%	2%	2.7%	0.5%	11.5%	2.4%	3.1%	1%	11.2%	2.3%	103.3%	110.9%	161.3%	125.2%	31.5%

Table (3-D)

<u>Table (3):-</u> Effect of steroid hormones and nonsteroid hormones on ApoA-1 dependent lipid effluxes:-

Influences of steroid hormones on basal, total and specific ApoA-1 dependent cholesterol (4-A, C) and phospholpids effluxes (4-B, D) in HepG2 cells were enumerated after stimulation with steroid hormones (4-A, C) "β-estradiol (100 nM for 36 h.), progesterone (1 uM for 60 h.), androgen (1 uM for 60 h.), dexamethasone (10^{-7} M for 16 h) and hydrocortisone (1 ug/ml for 24 h)" and non steroid hormones(4-B,D) "LH (5 ug for 16 h), somatostatin (2 ug/ml for 18 h), thyroxin (10 nM for 24 h), glucagon (5 nM for 8 h), insulin (100 nM for 18 h) and leptin (500 ng for 24 h)". Data were expressed as mean value in %, SD from three independent experiments. Specific ApoA-1 cholesterol and phospholipids effluxes of each experiment (Exp.1-3) were expressed in % mean of the three experiments and SD. * symbol means significant ($P \leq 0.05$).

Fig.5-A

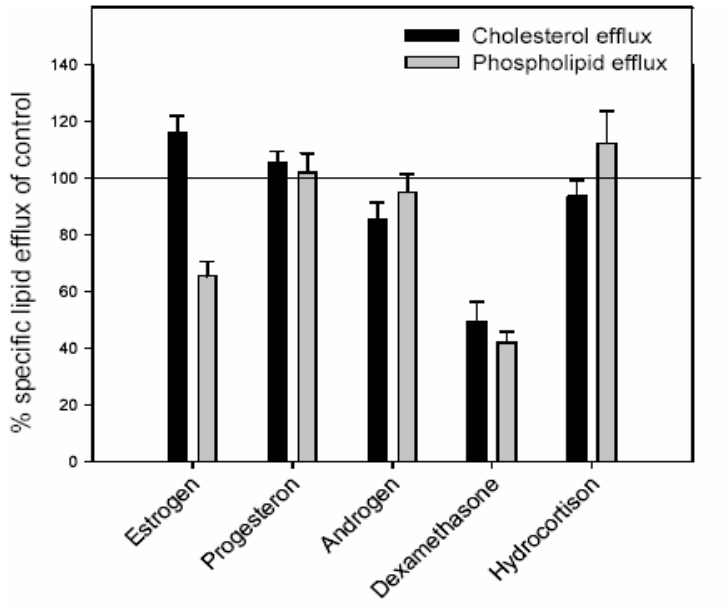

Fig.5-B

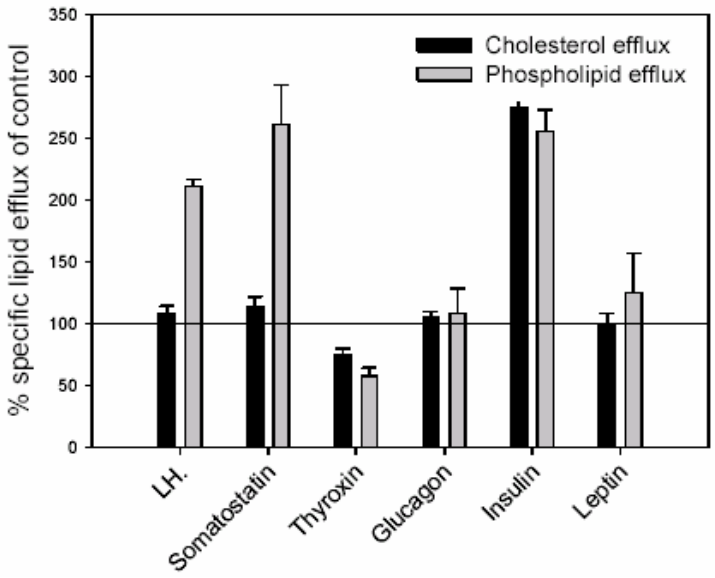

Fig.5. Effect of steroid and non steroid hormones on ApoA-1 dependent lipid effluxes in HepG2 cells:-
Influences of steroid hormones (fig.2-A) and non steroid hormones (fig.2-B) on specific ApoA-1 dependent cholesterol (black) and phospholpids effluxes (grey) in HepG2 cells were drawn in columns, vertical bars indicated SD, and the horizontal line passing through 100 % represented the specific ApoA-1 lipid effluxes without hormonal stimulation.

4.2 ABC transporter gene expression in human macrophages.

Since many of ABC transporters had been implicated in cellular lipid effluxes maily ABCA1, ABCA7, ABCG1, ABCG5 and ABCG8. All known ABC transporters mRNA expression were analysed by RT-PCR (Taqman) and these results were shown in table 4-A and B. To differentiate the expression levels of different ABC transporters, ΔCT values were calculated. The ranking of gene expression was as follow: ΔCT ≤12 indicated high expression, ΔCT 12-16 indicated intermediate expression, ΔCT 16-20 indicated low expression, and ΔCT >20 indicated no expression**.** The values of expression were calculated in % and considered as ≤ 50% as a downregulation and ≥ 150% as an upregulation.

Following M-CSF dependent differentiation of human monocytes to macrophages, ABCA1, ABCB2, ABCB3, ABCB6, ABCB8, ABCB9, ABCB10, ABCC1, ABCC3, ABCC5, ABCD1, ABCF2 and ABCF3 were highly expressed. ABCA2, ABCA3, ABCA5, ABCA7, ABCA12, ABCB1, ABCB4, ABCC4, ABCC6, ABCC7, ABCC9, ABCC10, ABCD2, ABCD3, ABCD4, ABCE1, ABCF1, ABCG1, and ABCG2 displayed intermediate expression. ABCA6, ABCA10, and ABCC2 were low expressed and the remaining ABC transporters were not expressed in these human cells.

In differentiated human macrophages, various hormones were added to the culture media according to the same conditions used for testing cholesterol and choline phospholipid effluxes. β-estradiol (100 nM for 36h) (Boffelli et al, 1999 and Kanamori et al, 2000) upregulated ABCC7 (172%), ABCC9 (155%), ABCD2 (164%), and ABCD3 (154%). Progesterone (1 μM for 60h) (Lemoisson et al, 1994) upregulated ABCA1 (276%), ABCA12 (202%), ABCB1 (159%), ABCB4 (154%), ABCC2 (180%), ABCC6 (193%), ABCC7

(250%), ABCC9 (244%), and ABCG1 (230%), and downregulated ABCA2 (40%) and ABCA7 (46%). Dihydrotestosterone (1 μM for 60h) (Faredin and Toth, 1975) upregulated ABCC10 (183%) and downregulated ABCA5 (29%), ABCA6 (7%), ABCA12 (3%), ABCB1 (7%), ABCB4 (49%), ABCC6 (13%), ABCC7 (12%), ABCD2 (6%), and ABCG2 (27%). Dexamethasone (10^{-7} M for 16h) (Dutta et al, 1989) upregulated ABCA3 (191%), ABCC7 (190%), ABCC9 (163%), ABCD2 (152.7%), and ABCG1 (159%), but downregulated ABCA2 (31%), ABCA10 (31%), ABCB3 (45%), ABCC1 (39%), ABCC3 (30%). Hydrocortisone (1 μg/ml for 24h) (Carr et al, 1981) upregulated ABCA3 (162%), and ABCC7 (188%), but downregulated ABCA2 (48%), ABCA5 (49%), ABCA6 (50%), ABCA10 (46%), ABCA12 (27%), ABCB1 (17%), ABCB3 (43%), ABCB4 (50%), ABCB8 (48%), ABCB9 (27%), ABCC6 (29%), ABCC9 (35%), ABCD2 (37%), and ABCG2 (30%) (Tab 4-A).

Triiodothyroxin (10 nM for 24h) (Stange et al, 1981) upregulated ABCC1 (156%), ABCC10 (174%), ABCD3 (220%), and ABCE1 (232%), but downregulated ABCA6 (27%), ABCA12 (8%), ABCB1 (16%), ABCB6 (8%), ABCC6 (15%), ABCC7 (14%), ABCC9 (13%), ABCD2 (26%), ABCG1 (31%), and ABCG2 (44%). Luteinizing hormone (LH) (5 μg/ml for 16h) (Magoffin, and Erickson, 1988) upregulated ABCB2 (185%), ABCB3 (220%), ABCB6 (171%), ABCC1 (247%), ABCC3 (185%), ABCC10 (203%), ABCD3 (237%), and ABCE1 (293%), but downregulated ABCA12 (47%), ABCB1 (42%), and ABCC6 (50%). Somatostatin (2 μg/ml for 18h) (Stange, et al, 1984) upregulated ABCA6 (185%), ABCA12 (156%), ABCB4 (150%), ABCC6 (170%), ABCC7 (226%), ABCC9 (218%), ABCC10 (150%), ABCD2 (181%), ABCD3 (220%), and ABCE1 (199%). Leptin (500ng/ml for 24h) (Berti et al, 1997) upregulated ABCA3 (206%), ABCA6 (230%), ABCA12 (217%), ABCB1 (174%), ABCB4 (155%), ABCB9 (156%), ABCC3 (150%), ABCC5 (153%), and ABCG2 (217%). Human recombinant insulin (100 nM for 18h) (Anne, et al 2000) upregulated ABCE1 (284%), and ABCG2 (330%) but downregulated ABCA5 (36%), ABCA6 (35%), ABCA12

(9%), ABCB1 (13%), ABCC6 (18%), ABCC7 (12%), and ABCD2 (15%). Glucagon (5 nM for 8h) (Anne et al, 2000) downregulated most of ABC transporters (Tab.4-B).

4.3 ABC transporter gene expression in HepG2 cells.

In HepG2 cells, ABCA1, ABCB1, ABCB2, ABCB3, ABCB4, ABCB10, ABCB11, ABCC1, ABCC2, ABCC5, ABCD3, ABCE1, ABCF1, ABCF2, ABCF3, ABCG5 and ABCG8 were highly expressed. ABCA2, ABCA3, ABCA5, ABCA7, ABCC3, ABCC4, ABCC6, ABCC10, ABCD1, ABCD4, ABCG1 and ABCG2 were intermediate expression. ABCA6, ABCA12, ABCC7, and ABCD2 were low expressed and the remaining ABC transporters were not expressed in these cells (table 4-C&D).

In HepG2 cells, one hormone was added to the culture media and incubated by the same concentration and for the same duration tested cholesterol and choline phospholipid effluxes.

β-estradiol upregulated ABCA6 (151%), ABCA12 (177%), ABCB1 (209%), ABCB11 (409%), ABCC7 (198%), ABCD1 (154%), ABCD2 (308%), ABCD3 (189%), ABCE1 (181%), ABCF1 (294%), ABCF2 (191%), ABCF3 (170%), and ABCG1 (155%) but downregulated ABCA2 (25%), ABCA7 (46.1%), and ABCG8 (40%). Progesterone upregulated ABCB11 (287%) and ABCC7 (180%). Androgen upregulated ABCB11 (229%). Dexamethasone upregulated ABCA3 (216%), and ABCB11 (647%), and ABCC7 (237%), but downregulated ABCA5 (36%), ABCA6 (10%), ABCA7 (32%), ABCA12 (16%), ABCB1 (50%), ABCB3 (40%), ABCB4 (46%), ABCC1 (34%), ABCC5 (37%), ABCD2 (9%), ABCD4 (43%), ABCG1 (35%), ABCG5 (45%), and ABCG8 (42%). Hydrocortisone upregulated ABCA6 (235%), ABCA12 (156%), ABCC7 (203%), ABCC10 (262%), and ABCD2 (197%), but downregulated ABCB3 (44%) (Tab.4-C).

Triiodothronine upregulated ABCB11 (356%), ABCC6 (158%), ABCD3 (203%), and ABCG5 (163%), but downregulated ABCA3 (31%), ABCA6 (31%), ABCA7 (35%), ABCB3 (43%), ABCC7 (36%), ABCD2 (48%), and ABCG1 (48%). LH upregulated ABCA5 (428%), ABCA6 (250%), ABCA7 (271%), ABCA12 (810%), ABCB2 (178%), ABCB3 (251%),

ABCB4 (197%), ABCB11 (332%), ABCC10 (172%), ABCD2 (561%), ABCD3 (191%), ABCD4 (150%), and ABCG8 (264%), but downregulated ABCB10 (20%). Somatostatin upregulated ABCA2 (187%), ABCA3 (187%), ABCA5 (415%), ABCA6 (243%), ABCA7 (364%), ABCA12 (600%), ABCB2 (186%), ABCB3 (270%), ABCB4 (205%), ABCB11 (422%), ABCC6 (201%), ABCC7 (739%), ABCC10 (165%), ABCD2 (513%), ABCD3 (158%), ABCD4 (189%), ABCF1 (171%), ABCF3 (197%), ABCG1 (197%), and ABCG8 (236%), and downregulated ABCB10 (30%). Leptin upregulated ABCA6 (192%), ABCA7 (181%), ABCC6 (171%), ABCC7 (181%), ABCC10 (177%), ABCD2 (174%), ABCD3 (169%), ABCD4 (161%), and ABCF1 (156%). Human recombinant insulin upregulated ABCA1 (197%), ABCB1 (150%), ABCB3 (150%), ABCA4 (151%), ABCB11 (392%), ABCC1 (244%), ABCC4 (290%), ABCC5 (170%), ABCD1 (204%), ABCD3 (303%), ABCF1 (224%), ABCF2 (191%), and ABCF3 (246%), but downregulated ABCA6 (30%), ABCC6 (45%), ABCC7 (9%), ABCC10 (4%), and ABCD2 (12%). Glucagon upregulated ABCA12 (160%), ABCB3 (193%), ABCB11 (393%), ABCC1 (221%), ABCC4 (204%), ABCD3 (412%), ABCE1 (183%), ABCF1 (212%), ABCF2 (158%), ABCF3 (157%), and ABCG2 (157%), downregulated ABCB4 (50%), ABCB10 (15%), ABCC6 (45%), ABCC7 (9%), and ABCC10 (4%) (Tab. 4-D).

	Mac.		*β-estradiol*			*Progesteron*			*Androgen*			*Dexamethasone*			*Hydrocortison*		
Conc.			*100nM*			*1uM*			*1uM*			*10-7M*			*1ug/ml*		
Dur.			*36h*			*60h*			*60h*			*16h*			*24h*		
	CT	***ΔCT***	***Value%***	***CT***	***ΔCT***	***Value%***	***CT***	***ΔCT***	***Value%***	***CT***	***ΔCT***	***Value %***	***CT***	***ΔCT***	***Value %***	***CT***	***ΔCT***
ABCA1	24.2	9.8	74%	25.1	10.7	276%	22.7	8.3	101%	24.2	9.8	100%	24.2	9.8	79.0%	24.6	10.2
ABCA2	29.8	15.4	89.7%	30	15.6	40.2%	31.1	16.7	100.7%	29.8	15.4	30.8%	31.5	17.1	48.3%	30.9	16.5
ABCA3	28.4	14.0	104.4%	28	13.6	127.8%	27.7	13.3	69%	28.6	14.2	190.5%	28.2	13.8	161.6%	ne	ne
ABCA4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne	ne	ne	ne	ne
ABCA5	28.2	13.8	100.6%	28.2	13.8	80%	28.5	14.1	28.7%	30	15.6	65.7%	28.8	14.4	48.6%	29.2	14.8
ABCA6	30.5	16.1	120%	30.4	16	141.3%	30.2	15.8	7%	34.5	20.1	140.6%	30.2	15.8	49.9%	31.7	17.3
ABCA7	27.3	12.9	80%	27.6	13.2	45.8%	28.4	14	58.1%	28.1	13.7	101.3%	27.3	12.9	131.5%	26.9	12.5
ABCA8	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCA9	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCA10	33.9	19.5	81.4%	34.2	19.8	57%	34.7	20.3	58%	34.7	20.3	31.1%	ne	ne	46%	ne	ne
ABCA12	28	13.6	144.7%	27.6	13.2	202%	27.1	12.7	2.6%	33.4	19	144.5%	27.6	13.2	27.1%	30	15.6
ABCB1	29.3	14.9	117%	29.1	14.7	159%	28.7	14.3	7%	32.9	18.5	96%	29.3	14.9	17%	31.7	17.3
ABCB2	22.4	8	84%	22.7	8.3	98%	22.5	8.1	80%	22.8	8.4	67%	23.2	8.8	58%	23.2	8.8
ABCB3	23.2	8.8	78%	23.6	9.2	71%	23.7	9.3	67%	23.8	9.4	45%	24.4	10	43%	24.4	10.0
ABCB4	28.5	14.1	107%	28.4	14	154%	27.5	13.1	49%	30.2	15.8	118%	28.1	13.7	50%	30.1	15.7
ABCB5	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB6	26.3	11.9	63%	27.1	12.7	60%	27.2	12.8	79%	26.9	12.5	60%	27.2	12.8	64%	27.1	12.7
ABCB7	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCB8	23.6	9.2	99%	23.6	9.2	61%	24.3	9.9	77%	23.9	9.5	51%	24.5	10.1	48%	24.6	10.2
ABCB9	25.6	11.2	110%	25.4	11	90%	25.7	11.3	51%	25.5	11.1	65%	26.1	11.7	27%	27.4	13
ABCB10	25.1	10.7	79%	25.5	11.1	55%	26	11.6	89%	25.3	10.9	52%	26.1	11.7	60%	25.8	11.4
ABCB11	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCC1	24.7	10.3	102.8%	24.6	10.2	113.6%	24.5	10.1	92.2%	24.8	10.4	39.4%	26	11.6	68.5%	25.2	10.8
ABCC2	32.5	18.1	89.1%	32.7	18.3	179.8%	31.7	17.3	92.8%	32.6	18.2	75%	32.9	18.5	117.5%	32.3	17.9
ABCC3	25.7	11.3	104.9%	25.6	11.2	116%	25.5	11.1	134.3%	25.3	10.9	30%	27.4	13	69.2%	26.2	11.8
ABCC4	27.2	12.8	106.9%	27.1	12.7	132%	26.8	12.4	77.2%	27.6	13.2	78.3%	27.6	13.2	67%	27.8	13.4
ABCC5	25	10.6	74.1%	25.4	11	111.1%	24.9	10.5	125.1%	24,7	10.3	72.5%	25.5	11.1	116.5%	24.8	10.4
ABCC6	28.3	13.9	124.6%	28	13.6	192.6%	27.4	13	12.6%	31.3	16.9	118.9%	28.1	13.7	28.9%	30.1	15.7
ABCC7	30.3	15.9	171.8%	29.5	15.1	249.8%	29	14.6	11.6%	33.4	19	190%	29.4	15	188%	29.5	15.1
ABCC8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCC9	29.3	14.9	154.6%	28.7	14.3	243.5%	28	13.6	99%	29.3	14.9	162.8%	28.6	14.2	34.1%	30.9	16.5
ABCC10	28.9	14.5	87.9%	29.1	14.7	110.9%	28.8	14.4	183%	28	13.6	60.2%	29.6	15.2	112.6%	28.7	14.3
ABCD1	26.1	11.7	108.3%	26	11.6	144.4%	25.6	11.2	130.6%	25.7	11.3	57.1%	26.9	12.5	54.6%	27	12.6
ABCD2	29.3	14.9	164%	28.6	14.2	142.4%	28.8	14.4	6.2%	33.3	18.9	152.7%	28.7	14.3	36.7%	30.8	16.4
ABCD3	29.7	15.3	154%	29.1	14.7	139.2%	29.3	14.9	132.8%	29.3	14.9	91%	29.8	15.4	141.7%	29.2	14.8
ABCD4	27.1	12.7	92%	27.2	12.8	135.4%	26.7	12.3	124.7%	26.8	12.4	79.3%	27.4	13	82.7%	27.4	13
ABCE1	28.3	13.9	112%	28.1	13.7	71.4%	28.8	14.4	121.7%	28	13.6	51.8%	29.3	14.9	52.7%	29.2	14.8
ABCF1	27.8	13.4	67%	28.4	14	92%	28	13.6	72%	28.3	13.9	67%	28.4	14	61%	28.5	14.1
ABCF2	26.4	12	55%	27.2	12.8	58%	27.2	12.8	87%	26.6	12.2	63%	27.1	12.7	52%	27.3	12.9
ABCF3	24.5	10.1	73%	25	10.6	96%	24.6	10.2	77%	25	10.6	73%	25	10.6	51%	25.6	11.2
ABCG1	27.5	13.1	93%	27.6	13.2	230.4%	26.4	12	93%	27.6	13.2	159%	26.8	12.4	96%	27.5	13.1
ABCG2	28.5	14.1	110%	28.3	13.9	127%	28.1	13.7	27%	30.4	16	102%	28.5	14.1	30%	30.2	15.8
ABCG4	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCG5	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCG8	Ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne

Table (4-A)

	Mac.		*L H.*			*Somatostatin*			*Triiodothyroxin*			*Glucagon*			*Insulin*			*Leptin*		
Conc.			*5ug/ml*			*2ug/ml*			*10nM*			*5nM*			*100nM*			*500ng/ml*		
Dur.			*16h*			*18h*			*24h*			*8h*			*18h*			*24h*		
	CT	*ΔCT*	*Value %*	*CT*	*ΔCT*	*Value %*	*CT*	*ΔCT*	*Value %*	*CT*	*ΔCT*	*Value %*	*CT*	*ΔCT*	*Value %*	*CT*	*ΔCT*	*Value %*	*CT*	*ΔCT*
ABCA1	24.2	9.8	95%	24.3	9.9	83%	24.5	10.1	70%	24.8	10.4	40%	25.6	11.2	85%	24.5	10.1	90%	24.4	10
ABCA2	29.8	15.4	75.3%	30.2	15.8	64%	30.4	16	92.6	29.9	15.5	49%	30.8	16.4	97.6%	29.8	15.4	92.8%	29.9	15.5
ABCA3	28.4	14	74%	28.5	14.1	146%	27.5	13.1	59%	28.8	14.4	68%	28.6	14.2	ne	28	13.6	206.4	27	12.6
ABCA4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCA5	28.2	13.8	88%	28.3	13.9	93%	28.3	13.9	54.6%	29	14.6	41%	29.5	15.1	35.6%	29.1	14.7	111.2%	28	13.6
ABCA6	30.5	16.1	78%	31	16.6	185%	29.8	15.4	27%	32.5	18.1	42.5%	31.9	17.5	34.5%	31.9	17.5	229.9%	29.5	15.1
ABCA7	27.3	12.9	60%	28	13.6	78%	27.6	13.2	88%	27.5	13.1	35.4%	28.4	14	119.6%	27.1	12.7	60.1%	28	13.6
ABCA8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCA9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCA10	33.9	19.5	108%	33.8	19.4	99%	33.9	19.5	84.8%	34.1	19.7	49%	34.9	20.5	95%	33.9	19.5	118%	33.6	19.2
ABCA12	28	13.6	46.5%	29.2	14.8	156.1%	27.5	13.1	7.9%	31.8	17.4	41%	29.4	15	8.6%	31.6	17.2	216.8%	27	12.6
ABCB1	29.3	14.9	42%	30.4	16	123%	29	14.6	16.1%	31.8	17.4	27%	31	16.6	13%	32.1	17.7	174%	28.5	14.1
ABCB2	22.4	8	185%	21.5	7.1	112%	22.3	7.9	84.5%	22.7	8.3	38%	23.8	9.4	89%	22.6	8.2	102%	22.4	8
ABCB3	23.2	8.8	220%	22.1	7.7	106%	23.1	8.7	97.9%	23.2	8.8	43%	24.4	10	84%	23.5	9.1	102%	23.2	8.8
ABCB4	28.5	14.1	58%	29.8	15.4	150%	27.4	13	105.4%	28.4	14	56%	29.5	15.1	68%	29.4	15	155%	27.5	13.1
ABCB5	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB6	26.3	11.9	171%	25.6	11.2	94%	26.5	12.1	8.2%	34.8	20.4	40%	27.7	13.3	91%	26.6	12.2	73%	26.9	12.5
ABCB7	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB8	23.6	9.2	102%	23.5	9.1	101%	23.6	9.2	107.7%	23,5	9.1	44%	24.8	10.4	97%	23.6	9.2	114%	23.4	9
ABCB9	25.6	11.2	69%	26.1	11.7	120%	25.2	10.8	85.1%	25.7	11.3	39%	26.9	12.5	66%	26.1	11.7	156%	24.9	10.5
ABCB10	25.1	10.7	97%	25.2	10.8	80%	25.4	11	104.8%	25	10.6	39%	26.5	12.1	101%	25.1	10.7	71%	25.6	11.2
ABCB11	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCC1	24.7	10.3	246.8%	23.4	9	110.7%	24.5	10.1	156.4%	24.1	9.7	39.3%	26	11.6	ne	24.5	10.1	121.5%	24.4	10
ABCC2	32.5	18.1	122.3%	32.2	17.8	126.7%	32.2	17.8	98.4%	32.6	18.2	55.9%	33.4	19	ne	32.3	17.9	107.3%	32.4	18
ABCC3	25.7	11.3	184.6%	24.8	10.4	91.7%	25.8	11.4	133.2%	25.3	10.9	40.8%	27	12.6	135%	25.3	10.9	149.5%	24.4	10
ABCC4	27.2	12.8	65.4%	27.8	13.4	124.1%	26.9	12.5	96.8%	27.3	12.9	49.3%	28.2	13.8	98.7%	27.2	12.8	103.2%	27.2	12.8
ABCC5	25	10.6	68%	25.6	11.2	92.7%	25.1	10.7	105.4%	24.9	10.5	43.7%	26.2	11.8	ne	24.6	10.2	152.7%	24.5	10.1
ABCC6	28.3	13.9	50%	29.3	14.9	169.8%	27.5	13.1	14.6%	31.1	16.7	44.2%	29.5	15.1	17.5%	30.8	16.4	122%	28	13.6
ABCC7	30.3	15.9	61.4%	31	16.6	225.6%	29.1	14.7	13.8%	33.2	18.8	52.3%	31.2	16.8	11.8%	33.4	19	124.1%	29.7	15.3
ABCC8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCC9	29.3	14.9	66.9%	29.9	15.5	218.2%	28.2	13.8	13.1%	32.3	17.9	54.1%	30.2	15.8	111%	29.1	18.1	123.5%	28.9	14.5
ABCC10	28.9	14.5	203.3%	27.9	13.5	150.3%	28.3	13.9	174%	28.1	13.7	62.7%	29.6	15.2	115.5	28.7	14.3	142.3%	28.6	14.2
ABCD1	26.1	11.7	112%	25.9	11.5	101.4%	26.1	11.7	108.4%	26	11.6	29.2%	27.9	13.5	105.6	26	11.6	107.3%	26	11.7
ABCD2	29.3	14.9	56.1%	30.1	15.7	181.2%	28.5	14.1	26.2%	31.2	16.8	44.4%	30.5	16.1	14.7%	32.1	17.7	103.2%	29.3	14.9
ABCD3	29.7	15.3	236.9%	28.5	14.1	219.7%	28.6	14.2	220%	28.6	14.2	38%	31.1	16.7	ne	28.7	14.3	122%	29.5	15.1
ABCD4	27.1	12.7	106.3%	27	12.6	119.1%	26.9	12.5	101.3%	27.1	12.7	32.5%	28.7	14.3	100%	27.1	12.7	124.1%	26.8	12.4
ABCE1	28.3	13.9	292.8%	26.8	12.4	199.4%	27.3	12.9	232.2%	27.1	12.7	69.2%	28.8	14.4	284%	26.8	12.4	132.5%	27.9	13.5
ABCF1	27.8	13.4	136%	27.4	13	87%	28	13.6	96.1%	27.9	13.5	39%	29.1	14.7	94%	27.9	13.5	86%	28.1	13.7
ABCF2	26.4	12	114%	26.2	11.8	86%	26.6	12.2	107.1%	26.3	11.9	43%	27.6	13.2	112%	26.2	11.8	88%	26.6	12.2
ABCF3	24.5	10.1	110%	24.4	10	94%	24.7	10.3	93%	24.7	10.3	59%	25.4	11	96%	24.6	10.2	103%	24.5	10.1
ABCG1	27.5	13.1	81%	27.8	13.4	114%	27.3	12.9	31.4%	29.1	14.7	42%	28.7	14.3	137%	28.7	14.3	148%	26.9	12.5
ABCG2	28.5	14.1	62%	29.2	14.8	141%	28	13.6	44.2%	29.7	15.3	41%	29.7	15.3	330%	29.6	15.2	217%	27.4	13
ABCG4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCG5	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCG8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne

Table (4-B)

	HepG2		Estrogen			Progesteron			Androgen			Dexamethasone			Hydrocortison		
Conc.			100nM			1uM			1uM			10-7M			1ug/ml		
Dur.			36h			60h			60h			16h			24h		
	CT	ΔCT	Value%	CT	ΔCT	Value%	CT	ΔCT	Value%	CT	ΔCT	Value%	CT	ΔCT	Value%	CT	ΔCT
ABCA1	24.6	9.8	71.2%	25.2	10.4	111.3%	24.5	9.7	102.9%	24.6	9.8	64.6%	25.5	10.7	103.5%	24.6	9.8
ABCA2	29.2	14.4	25%	31.8	17	93.9%	29.2	14.4	97.1%	29.2	14.4	69.4%	31.4	16.6	131.8%	28.8	14
ABCA3	28.4	13.6	114.4%	28.3	13.5	94.8%	28.4	13.6	91%	28.4	13.6	215.6%	31.6	16.8	131.8%	28.2	13.4
ABCA4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCA5	27.6	12.8	97.5%	27.7	12.9	101.7%	27.6	12.8	91.8%	27.7	12.9	36%	29.5	14.7	101.3%	27.6	12.8
ABCA6	32.9	18.1	151.1%	32.3	17.5	131.3%	32.3	17.5	83%	33.1	18.3	9.5%	36.5	21.7	234.5%	31.1	16.3
ABCA7	28	13.2	46.1%	29.3	14.5	91%	28.2	13.4	90.1%	28.2	13.4	31.6%	29.8	15	113.7%	27.8	13
ABCA8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCA9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCA10	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	Ne
ABCA12	31.9	17.1	177%	29.8	15	115.5%	30.5	15.7	64.2%	31.7	16.9	15.7%	34	19.2	156.2%	29.9	15.1
ABCB1	24.7	9.9	209%	23.4	8.6	105.1%	24.7	9.9	115.4%	24	9.2	49.8%	25.7	10.9	82%	25	10.2
ABCB2	24.4	9.6	95%	24.9	10.1	85.2%	24.3	9.5	106.6%	24.3	9.5	54%	25.8	11	19%	24.1	9.3
ABCB3	24.3	9.5	108%	24.2	9.4	81.2%	24.6	9.8	91.7%	24.5	9.7	40%	24.2	9.4	44%	24.4	9.6
ABCB4	24.6	9.8	131%	25	10.2	104.2%	24.6	9.8	114.1%	24.8	10	45.5%	25.3	10.5	117%	24.8	10
ABCB5	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB6	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB7	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCB10	26.4	11.6	138%	26.1	11.3	108%	26.3	11.5	108%	26.3	11.5	70%	27.2	12.4	92%	26.6	11.8
ABCB11	26.3	11.5	409%	25.7	10.9	286.9%	25.5	10.7	229.4%	25.5	10.7	646.8%	25.8	11	115.8%	26.3	11.5
ABCC1	24.8	10	144%	24.6	9.8	94.2%	24.7	9.9	108.7%	24.7	9.9	34.1%	27.2	12.4	80%	25	10.2
ABCC2	24	9.2	85%	24.5	9.7	110%	23.8	9	102.4%	24	9.2	79.3%	24.7	9.9	82.4%	24.5	9.7
ABCC3	26.9	12.1	64%	28.6	13.8	107.7%	26.7	11.9	100.7%	26.9	12.1	70.6%	28.4	13.6	92.5%	27.1	12.3
ABCC4	28.1	13.3	125%	27.1	12.3	102.4%	28.1	13.3	101.7%	28.1	13.3	112.2%	27.5	12.7	90%	28.2	13.4
ABCC5	26.3	11.5	127%	25.6	10.8	90%	26.6	11.8	100.2%	26.3	11.5	37%	27.7	12.9	92.2%	26.6	11.8
ABCC6	27	12.2	59%	28.3	13.5	119.7%	26.5	11.7	99.6%	27	12.2	93.7%	27.8	13	89%	27.3	12.5
ABCC7	32.7	17.9	198%	32.1	17.3	179.9%	32.2	17.4	52.6%	34	19.2	237%	31.8	17	202.9%	32.1	17.3
ABCC8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCC9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCC10	28.1	13.3	137%	27.8	13	88%	28.2	13.4	79.4%	28.3	13.5	72.7%	28.4	13.6	262.3%	26.1	11.3
ABCD1	27.1	12.3	154%	26.6	11.8	98.2%	27.1	12.3	86.1%	27.2	12.4	117.4%	26.9	12.1	75.8%	27.3	12.5
ABCD2	32.1	17.3	308%	31.4	16.6	119.9%	32.2	17.4	69.1%	33.5	18.7	9.4%	ne	ne	197%	31.1	16.3
ABCD3	25.7	10.9	189%	25.6	10.8	101.8%	25.7	10.9	89.6%	26.3	11.5	138%	25.7	10.9	76.3%	26.6	11.8
ABCD4	27.1	12.3	111%	27	12.2	98.7%	27.2	12.4	87.7%	27.3	12.5	43.2%	28.2	13.4	87.9%	27.2	12.4
ABCE1	24.5	9.7	181%	24.4	9.6	94.4%	24.6	9.8	97.5%	24,6	9,8	89.1%	24.7	9.9	65.4%	25.7	10.9
ABCF1	26.4	11.6	294%	25.7	10.9	98.2%	26.4	11.6	76.8%	26.8	12	67.2%	27.2	12.4	55.3%	28	13.2
ABCF2	25.4	10.6	191%	25.2	10.4	87.9%	25.6	10.8	80.1%	26.3	11.5	81%	26.3	11.5	63.5%	26.5	11.7
ABCF3	25.9	11.1	170%	25.7	10.9	84%	25.9	11.1	83.4%	25.9	11.1	111%	25.8	11	75.8%	26.1	11.3
ABCG1	28.8	14	155%	26.8	12	90.9%	29.3	14.5	67.8%	31.2	16.4	34.5%	32	17.2	75.8%	30.4	15.6
ABCG2	29.6	14.8	66%	30	15.2	117.1%	29.3	14.5	119.2%	29.3	14.5	80.8%	29.8	15	91%	29.7	14.9
ABCG4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCG5	25.3	10.5	52%	26.7	11.9	108.6%	25.1	10.3	92%	25.4	10.6	45%	26.8	12	101.4%	25.3	10.5
ABCG8	25.9	11.1	40%	27.5	12.7	66.6%	26.3	11.5	67.9%	26.3	11.5	42.1%	27.5	12.7	111.6%	25.9	11.1

Table (4-C)

	HepG2		L H.			Somatostatin			Triiodothyroxin			Glucagon			Insulin			Leptin		
Conc.			5ug/ml			2ug/ml			10nM			5nM			100nM			500ng/ml		
Dur.			16h			18h			24h			8h			18h			24h		
	CT	ΔCT	Value %	CT	ΔCT	Value %	CT	ΔCT	Value %	CT	ΔCT	Value %	CT	ΔCT	Value %	CT	ΔCT	Value %	CT	ΔCT
ABCA1	24.6	9.8	114.3%	24	9.6	118.1%	24.3	9.5	105.7%	24.6	9.8	109%	24.6	9.8	197%	23.4	8.6	104.9%	24.6	9.8
ABCA2	29.2	14.4	120.7%	29	14.2	187%	28.6	13.8	85.2%	29.3	14.5	106%	29.1	14.3	83.5%	29.3	14.5	115.2%	29	14.2
ABCA3	28.4	13.6	120.7%	28.3	13.5	187%	28.1	13.3	31.2%	30.2	15.4	62%	28.6	13.8	92.8%	28.4	13.6	115.2	28.3	13.5
ABCA4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCA5	27.6	12.8	428%	26	11.2	415%	26	11.2	55.4%	28.4	13.6	142%	27.4	12.6	99.3%	27.6	12.8	132.6%	27.5	12.7
ABCA6	32.9	18.1	250.2%	30.6	15.8	243.4%	30.6	15.8	31.4%	34.6	19.8	145.1%	32.3	17.5	30.4%	34.7	19.9	191.6%	31.3	16.5
ABCA7	28	13.2	271%	25	10.5	364%	24.1	9.3	35%	29.7	14.9	97.5%	28	13.2	60.9%	28.9	14.1	180.7%	26.1	11.3
ABCA8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCA9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCA10	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCA12	31.9	17.1	810.1%	27	11.9	600.3%	27.1	12.3	56.7%	31.6	16.8	160%	29.9	15.1	97.4%	31.9	17.1	121.5%	30.4	15.6
ABCB1	24.7	9.9	104.2%	25	9.9	130.2%	23.9	9.1	62.5%	25.2	10.4	109.3%	24.7	9.9	150%	23.7	8.9	105.8%	24.7	9.9
ABCB2	24.4	9.6	177.9%	23	7.9	185.9%	22.6	7.8	56.3%	25.8	11	140%	23.8	9	109.2%	24.2	9.4	102.7%	24.4	9.6
ABCB3	24.3	9.5	251%	22	6.8	270%	21.4	6.6	43%	24.2	9.4	193%	22	7.2	149.6%	22.3	7.5	112.6%	24	9.2
ABCB4	24.6	9.8	197.3%	24	9.1	204.7%	23.8	9	114.6%	24.9	10.1	49,9%	25.2	10.4	151%	25.2	10.4	122.6%	24.9	10.1
ABCB5	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCB6	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCB7	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCB8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCB9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCB10	26.4	11.6	20%	28	13.4	30%	27.9	13.1	99%	26.4	11.6	15%	29.1	14.3	94%	26.6	11.8	114%	26.2	11.4
ABCB11	26.3	11.5	332.4%	26	11.1	422%	24.8	10	355.6%	25.9	11.1	393%	25.8	11	392%	25.8	11	138.4%	25.3	10.5
ABCC1	24.8	10	79.2%	25	10.5	56%	26.3	11.5	63.5%	26	11.2	221.2%	24.3	9.5	244.3%	24.2	9.4	117.9%	24.7	9.9
ABCC2	24	9.2	75.2%	25	9.9	96%	24.3	9.5	125.7%	23.5	8.7	117.3%	23.7	8.9	140.2%	23	8.2	124.9%	23.5	8.7
ABCC3	26.9	12.1	95.2%	27	12.3	82%	27.5	12.7	75.9%	28	13.2	81.2%	27.7	12.9	74.6%	28.1	13.3	101.9%	26.9	12.1
ABCC4	28.1	13.3	63.2%	29	14.1	60.9%	28.9	14.1	122.2%	27.1	12.3	204%	26.2	11.4	289.4%	25.6	10.8	105.9%	28	13.2
ABCC5	26.3	11.5	110.7%	26	11.2	128.7%	25.4	10.6	75.6%	26.7	11.9	102.6%	26.3	11.5	169.7%	24.9	10.1	117.3%	25.9	11.1
ABCC6	27	12.2	124.6%	27	12	200.8%	23.9	9.1	157.9%	26.2	11.4	44.6%	28	13.2	44.6%	28	13.2	170.9%	24.6	9.8
ABCC7	32.7	17.9	103%	29	13.8	738.8%	29	14.2	35.7%	ne	ne	8.7%	n.e.	n.e.	8.7%	n.e.	n.e.	181.1%	32.3	17.5
ABCC8	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCC9	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	ne	ne	ne
ABCC10	28.1	13.3	172.1%	26	11.6	165.4%	26.3	11.5	62.1%	28.5	13.7	3.7%	30.3	15.5	3.7%	30.3	15.5	177.3%	26.5	11.7
ABCD1	27.1	12.3	115.3%	27	12.1	126.6%	26.8	12	98%	27.1	12.3	130.4%	26.8	12	204%	25.9	11.1	108.8%	27	12.2
ABCD2	32.1	17.3	560.6%	29	14.1	513.2%	29	14.2	47.6%	ne	ne	115.4%	32.2	17.4	11.6%	n.e.	n.e.	174.1%	31.0	16.2
ABCD3	25.7	10.9	190.7%	26	10.8	157.5%	25.6	10.8	202.6%	25.6	10.8	411.5%	24.4	9.6	303.1%	24.6	9.8	168.6%	25.6	10.8
ABCD4	27.1	12.3	150.4%	27	12.1	188.7%	26.5	11.7	56.8%	28	13.2	84.7%	27.3	12.5	81.9%	27.3	12.5	160.7%	26.8	12
ABCE1	24.5	9.7	115.3%	25	9.8	126.5%	24.6	9.8	108.8%	24.5	9.7	182.6%	24.4	9.6	59.2%	26.1	11.3	138.2%	24.5	9.7
ABCF1	26.4	11.6	115.3%	27	11.7	170.6%	25.9	11.1	65.5%	27.2	12.4	211.7%	25.8	11	223.5%	25.8	11	155.7%	26	11.2
ABCF2	25.4	10.6	115.3%	26	10.7	124.7%	25.5	10.7	73.7%	26.7	11.9	158.2%	25.3	10.5	190.8%	25.2	10.4	131%	25.3	10.5
ABCF3	25.9	11.1	115.3%	26	11	197.4%	25.4	10.6	107%	25.9	11.1	156.9%	25.8	11	245.7%	24.8	10	99.9%	25.9	11.1
ABCG1	28.8	14	115.3%	28	13	197.4%	25.7	10.9	48%	32.8	18	117.4%	27.8	13	90.5%	32.3	17.5	99.9%	28.8	14
ABCG2	29.6	14.8	102.8%	30	14.8	96.5%	29.7	14.9	65.5%	30	15.2	156.5%	28.8	14	124.9%	29	14.2	85.2%	29.8	15
ABCG4	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne	ne
ABCG5	25.3	10.5	130.9%	25	10.2	137.2%	24.9	10.1	162.5%	24.9	10.1	63%	26.6	11.8	87.2%	25.9	11.1	108.1%	25.1	10.3
ABCG8	25.9	11.1	263.5%	25	9.9	235.8%	24.9	10.1	76.3%	26.1	11.3	76.5%	26.1	11.3	121.2%	25.9	11.1	115.3%	25.9	11.1

Table (4-D)

Table (4):- Effect of Steroid hormones on ABC transporters mRNA expression:-
ABC gene expression has been measured in human macrophage (3-A,B) and HepG2 cells (3-C,D) following stimulation with steroid hormones (3-A,C) ''β-estradiol (100 nM for 36 h), progesterone (1 uM for 60 h.), androgen (1 uM for 60 h), dexamethasone(10^{-7} M for 16 h) and hydrocortisone (1 ug/ml for 24 h)'' or with non steroid hormones(3-B,D) ''LH (5 ug for 16 h), somatostatin (2 ug/ml for 18 h), thyroxin (10 nM for 24 h), glucagon (5 nM for 8 h), human recombinant insulin (100 nM for 18 h) and leptin (500 ng for 24 h)''. ABC transporters mRNA levels were determined by TaqMan real time RT-PCR. Values are presented as mean of triplicated value in % comparing with non stimulated cells, CT and ΔCT (it is the difference between CT of ABC and 18SrRNA as a reference gene). Upregulated gene expressions (≥ 150%) were coloured their background with red and downregulated gene expressions (≤ 50%) were coloured their background with blue.

4.4 Effect of insulin concentration on ABCA1 in human macrophages.

Different insulin hormone concentrations (25nM, 50nM, 100nM, 150nM, 200nM, 250nM and 300nM) were added to human macrophages cultured in Macrophage-SFM medium with M-CSF and incubated for 3 h. Insulin upregulated ABCA1 gene expression at 25 nM (153% ± 3.8%), 50 nM (164% ± 8.4%), 100 nM (195% ± 8.9%) and 250 nM (156% ± 4%) with its maximum influence at 150 nM insulin concentration (217% ± 6.7%) (Tab. 5) and (Fig.6).

	ABCA1	
Insulin Conc.	Value %	SD %
25nM	153.4*	3.8
50nM	164.2*	8.4
100nM	195.3*	8.9
150nM	216.8*	6.7
200nM	186.9	11.9
250nM	156.4*	4
300nM	169.4	15.8

Table (5):- Concentration schedule of insulin on ABCA1.
Human macrophages were incubated with variable insulin concentrations (25, 50, 100, 150, 200, 250, 300 nM) for 3h. ABCA1 mRNA levels were determined by TaqMan real time RT-PCR. Values are presented as mean of triplicate value in % compared to HepG2 cells and SD. * symbol means significant ($P \leq 0.05$).

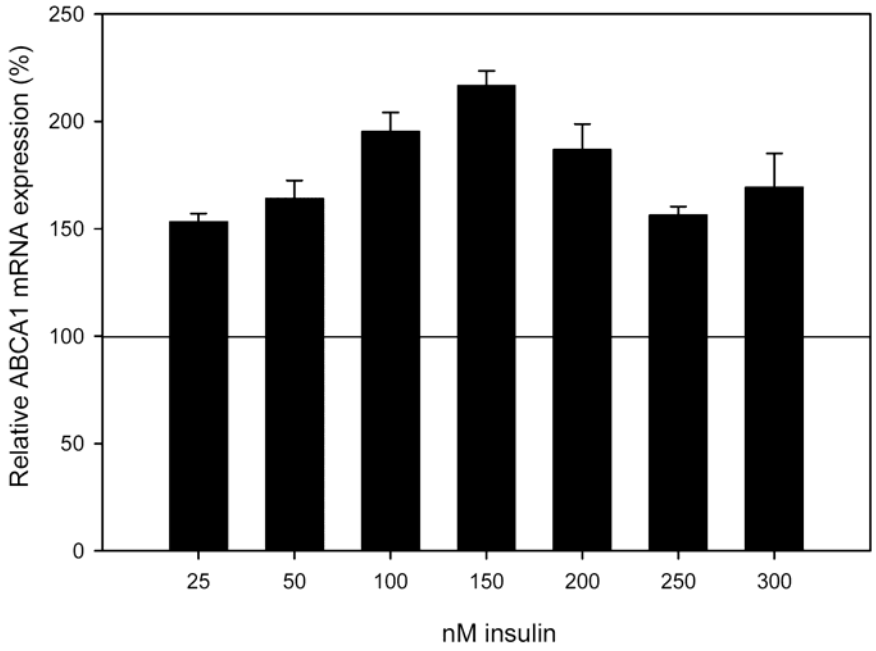

Fig. 6. ABCA1- expression as a function of insulin concentration.
% of relative of ABCA1 mRNA expressions in human macrophages had been drawn in column form and vertical bars indicated SD, were compaired in different insulin concentration (0, 25, 50, 100, 150, 200, 250, and 300 nM) for 3 h insulin incubation.

4.5 Time kinetic of insulin on ABCA1, ABCG1 and ABCA7 genes expression in macrophages.

The insulin impact on ABCA1, ABCG1 and ABCA7 genes expressions in a time dependent manner was measured by TaqMan real time RT-PCR and the effect on ABCA1 protein expression was detected by western blot. 150 nM insulin was added to macrophages and incubated for 1h, 2h, 3h, 4h, 6h, 12h, and 24 h. Insulin significantly upregulated ABCA1 mRNA expression in a time dependent manner starting after the first hour (141% $\pm$ 8.8%) and peaking after 3h (220% $\pm$ 11.8%). A similar significant upregulation could be seen with ABCG1 which started after the first hour (135% $\pm$ 5.6%) and peaked after 3 h of insulin incubation (231% $\pm$ 14.5%). ABCA7 was not affected by human insulin (Tab.6 and Fig.7). To compare the influence of insulin on ABCA1 gene expression with ABCA1 protein expression, western blot was done in a time schedule (1h, 2h, 3h, 4h, 6h, 12h, 24h and 48h) of insulin incubation (150 nM) in human macrophages. We found that, high ABCA1 protein expression observed after 1 h and extended until 12 h with maximum intenisty of the band after 4 h of

insulin stimulation. However, glucagon supressed ABCA1 protein expression (the ABCA1 protein band was only faint). ABCA1 protein expression is standardized by β-actin (Fig.8).

Insulin Duration	**ABCA1**		**ABCG1**		**ABCA7**	
	Value %	SD %	Value %	SD %	Value %	SD %
1h.	141.4*	8.8	135.4*	5.6	82.4*	3.6
2h.	173.6*	9.4	185.4*	10.4	98.5	6.7
3h.	220.3*	11.8	231.1*	14.5	80.7*	3.9
4h.	135.7*	7.8	134.7*	8.3	82.9	7.8
6h.	105.4	10.9	123.9*	6.9	86.9	6.1
12h.	95.8	11.2	125*	16.3	78*	2.9
24h.	89.6*	5.9	111.4*	12.5	123.1*	5.1

Table (6):- Time kinetic of insulin on ABCA1, ABCG1 and ABCA7 gene expressions.
Expression of ABCA1, ABCG1 and ABCA7 genes has been measured in human macrophage following stimulation with insulin (150nM) for 1, 2, 3, 4, 6, 12, 24 h. ABC mRNA levels were determined by TaqMan real time RT-PCR values are presented as mean of triplicate values in % comparing with non and SD. * symbol means significant ($P \leq 0.05$).

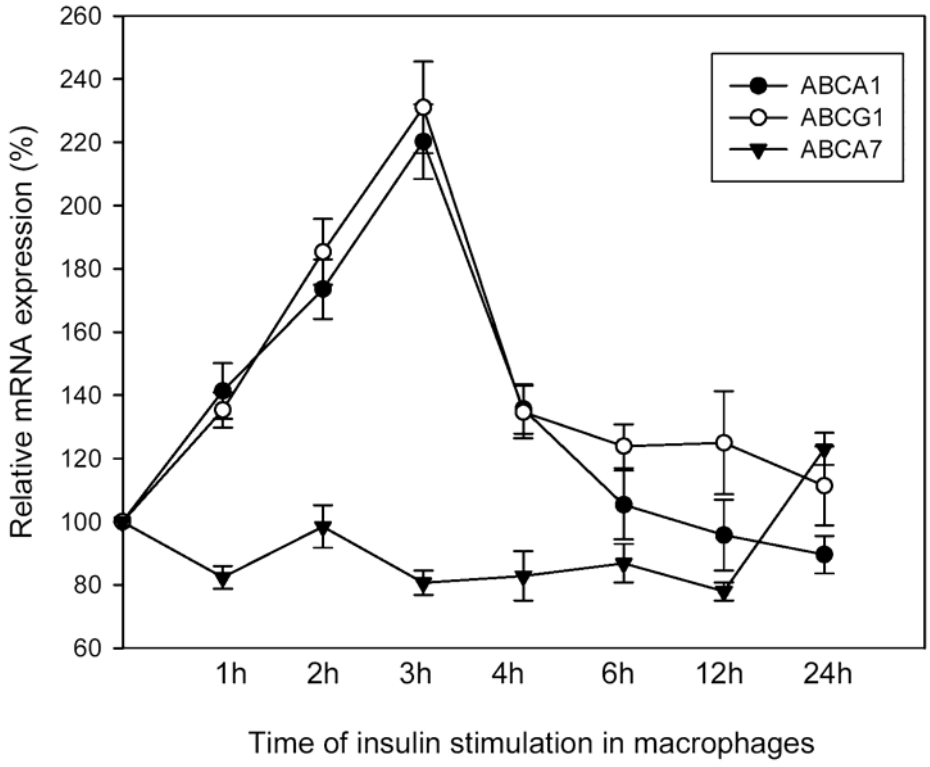

Fig. 7. Time kinetic of insulin on ABCA1, ABAG1 and ABCA7 gene expressions in macrophages.
% of relative of ABCA1 (•), ABCG1 (o) and ABCA7 (▼) mRNA expressions in human macrophage had been drawn in linear scale with vertical bars indicated SD in relation to duration of insulin stimulation (150 nM) for 1, 2, 3, 4, 6, 12, 24 h.

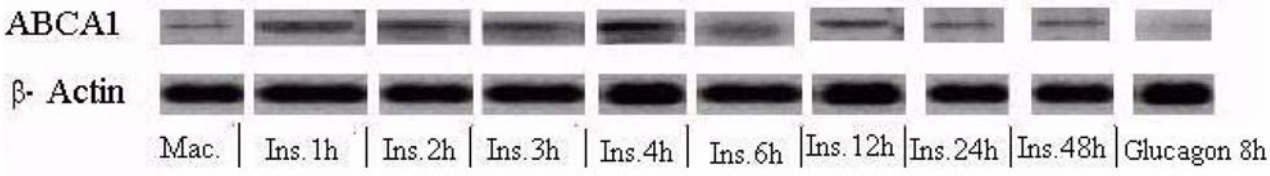

Fig.8. Kinetics of insulin on ABCA1 protein expression in macrophages.
Variable duration (1, 2, 3, 4, 6, 12, 24, and 48 h) of 150nM insulin or glucagon (5 nM for 8 h) on ABCA1 protein expression of human macrophage was tested by western blot. β-actin was used as a standard protein.

4.6 Time kinetic of insulin on ABCA1 gene expression in HepG2 cells.

To study the impact of human recombinant insulin on ABC transporter expression in HepG2 cells, insulin (150 nM)is added to the cells for 1h, 2h, 3h, 4h, 6h, 9h, 12h, 18h and 24h. ABCA1 gene expression was detected by TaqMan real time PCR. Insulin upregulated ABCA1 gene expression which started after 1h (124% ± 2.1%) and peaked after 3 h (150.3% ± 10.5%) then gradually declined and then peaked again after 18 hours (230% ±15.9%). So ABCA1 gene expression in HepG2 cells had two peaks with insulin after 3 and 18 h of insulin incubation (Tab.7 and Fig.9).

Insulin Duration	**ABCA1**	
	Value %	SD %
1h.	123.5*	2.1
2h.	139.3*	7.2
3h.	150.3*	10.5
4h.	138.4*	7.9
6h.	132.1	11.2
9h.	185.3	12.4
12h.	197.6*	10.1
18h.	230.3*	15.9
24h.	128.9*	7.4

Table (7):- Time kinetic of insulin on ABCA1 gene expression.
Expression of ABCA1 gene has been measured in HepG2 cells following incubation with insulin (150 nM) for 1, 2, 3, 4, 6, 9, 12, 18, 24 h. ABCA1 mRNA levels were determined by TaqMan real time RT-PCR values are presented as mean of triplicate values in % comparing with HepG2 cells and SD. * symbol means significant ($P \leq 0.05$).

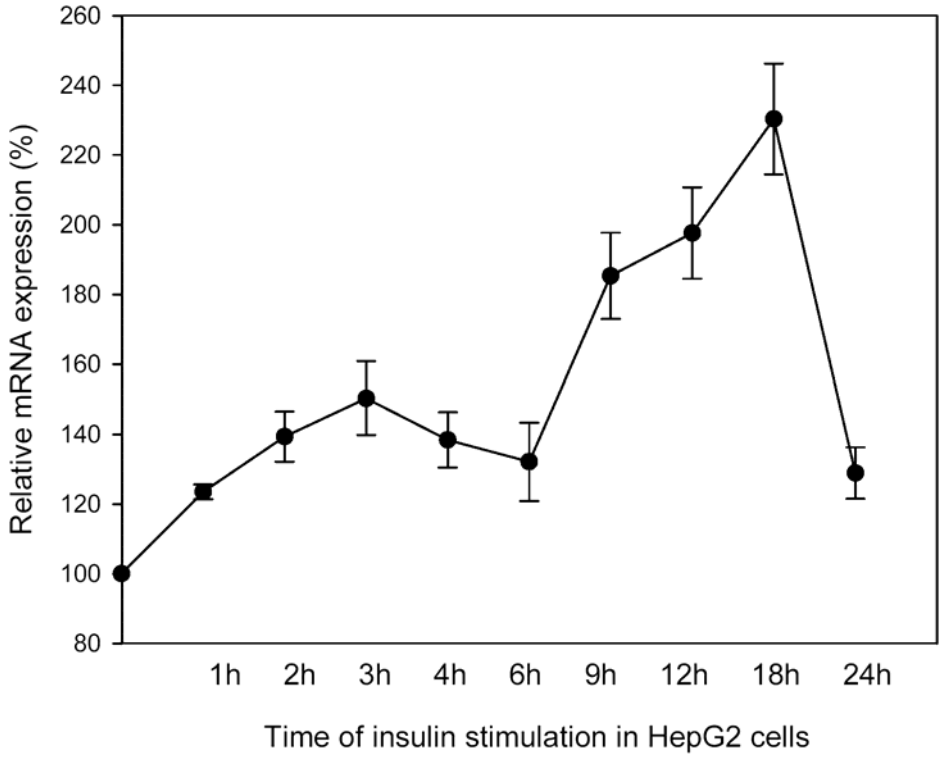

Fig. 9. Kinetics of insulin on ABCA1 gene expression in HepG2 cells.
% of relative of ABCA1 (•) mRNA expressions in HepG2 cells had been drawn in linear scale with vertical bars indicate SD in relation to duration of insulin stimulation (150 nM) for 1, 2, 3, 4, 6, 9, 12, 18, 24 h.

4.7 Insulin stimulates ABCA1 expression via the MAP kinase pathway.

Since the insulin pathway could involve different signalling mechanisms (Shephered et al, 1995), the influence of specific kinase inhibitors on insulin dependent ABCA1 expression was analyzed. Human macrophages were preincubated for 1 h with Wortmannin (PI-3 kinase inhibitor; 100 nM) or PD98059 (selective MEK-1/2 inhibitor; 25μM) before stimulation with human insulin (150 nM) for additional 3 hours (Lida el al, 2001). Insulin significantly upregulated ABCA1 mRNA expression (250% ± 14.4%). Pretreatment with PD98059 completely abolished insulin ABCA1 gene induction, while Wortmannin did not antagonize upregulation of ABCA1 gene expression by insulin (Tab.8) and (Fig.10). This can also be observed at the protein level, where the insulin augmented ABCA1 protein expression which was strongly abolished by pretreatment with PD98059 in human macrophages but not

antagonized by pretreatment with Wortmannin. ABCA1 protein expression is standardized by β-actin protein expression (Fig.11).

	ABCA1	
	Value%	SD%
Macrophage+insulin	250*	14.4
Macrophage+insulin +Wortmann	245*	12.3
Macrophage+insulin+ PD98059	91*	7.2

Table (8):- Role of insulin with or without kinase inhibitors on ABCA1 gene expression. Human macrophages were preincubated with the specific PI-3 kinase inhibitor Wortmannin (100 nM) or the MAP kinase inhibitor PD98059 (25 μM) for 1h before incubation with insulin for further 3h. ABCA1 mRNA expression was determined by TaqMan real time RT-PCR. Values are presented as mean of triplicated values in % compaired with macrophages and SD. * symbol means significant ($P \leq 0.05$).

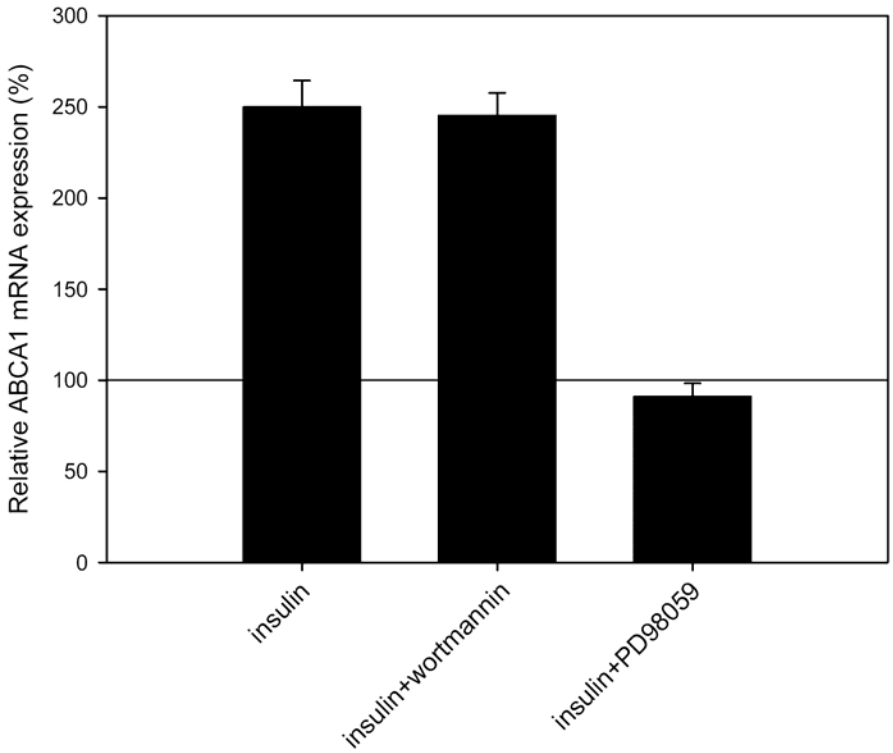

Fig. 10. Role of insulin with or without kinase inhibitors on ABCA1 gene expression. % of relative of ABCA1 mRNA expressions in human macrophage had been drawn in column form with vertical bars indicate SD in relation to insulin (150 nM for 3h; first column) and with preincubated in Wortmannin (100nM for 1h; second column) or PD98059 (25μM for 1h; third column).

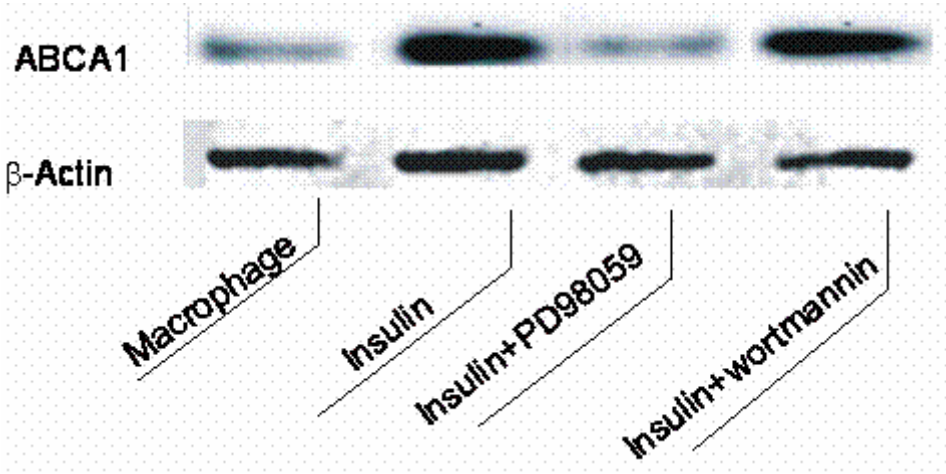

Fig.11. Role of insulin with or without kinase inhibitors on ABCA1 protein expression. Insulin (150nM for 3 hours) influence on ABCA1 protein expression of human macrophages was tested by western blot, compared with that after preincubated with the specific MAP kinase inhibitor (PD98059; 25 μM) or PI-3 kinase inhibitor (wortmannin; 100 nM) for 1h before insulin incubation. β-actin was used as a standard protein.

4.8 Time kinetic of β-estradiol on ABCA1 and ABCG1 genes expression in human macrophages.

100 nM β-estradiol is added to macrophages and incubated for 1, 3, 6, 12, 18, 24, and 36 h. β-estradiol upregulated ABCA1 gene expression significantly in a time dependent manner, which started after the first hour (175% ± 7% $p < 0.05$) untill the twelfth hour of incubation (131.7% ±2.8% $p < 0.05$) and peaked after the third hour of incubation (218% ± 8.9% $p < 0.05$) (Tab.9 and Fig.12). A similar upregulation could be seen for ABCG1 which started after the first hour (235% ± 13.6% $p < 0.05$) till the twenty fourth hour of incubation (137% ± 9.3% $p < 0.05$) and peaked after the third hour of estrogen incubation (465% ± 18.5% $p < 0.05$). ABCA7 was not affected by estrogen (Tab.9) and (Fig.12).

β-estradiol duration	ABCA1		ABCG1		ABCA7	
	Value %	SD %	Value %	SD %	Value %	SD %
1h.	174.6*	7	234.5*	13.6	98.3*	3.4
3h.	218.4*	8.9	465.1*	15.2	96.4*	5.2
6h.	147.1*	5.1	195.8*	12.8	84.8	12.1
12h.	131.7*	2.8	141.8*	6.8	88.7*	3.1
18h.	102	15.7	132.6	5.2	81.9*	4.9
24h.	124.2*	7.1	136.5*	2.8	95	6.8
36h.	73.6*	7.8	93.4	3.4	80.1	7.1

Table (9):- Time kinetic of β-estradiol on ABCA1, ABCG1 and ABCA7 genes expression.

Expression of ABCA1, ABCG1 and ABCA7 genes had been measured in human macrophages following stimulation with β-estradiol (100nM) for 1, 3, 6, 12, 18, 24 and 36 h. ABC mRNA levels were determined by TaqMan real time RT-PCR. Values are presented as mean of triplicated values in % compared with and SD. * symbol means significant ($P \leq 0.05$).

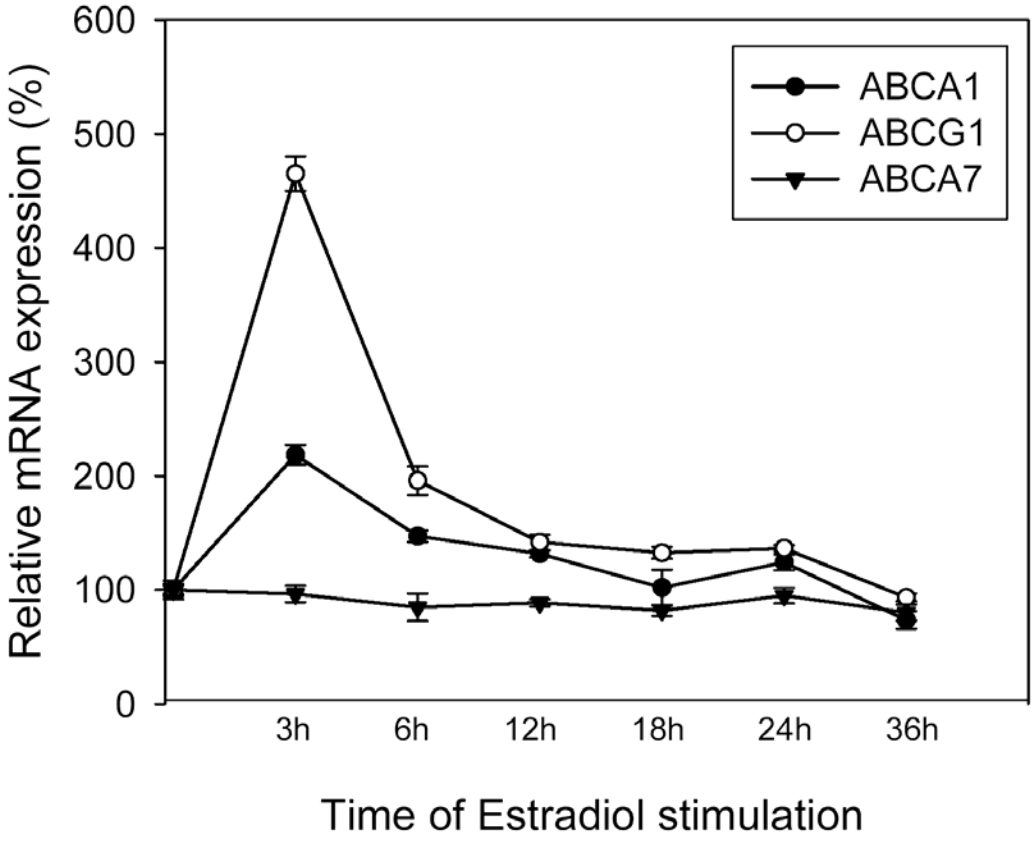

Fig. 12. Time kinetic of β-estradiol on ABCA1, ABCG1 and ABCA7 genes expression.

% of relative of ABCA1 (•), ABCG1 (o) and ABCA7 (▼) mRNA expressions in human macrophage had been drawn in linear scale with vertical bars indicate SD in relation to variable duration of β-estradiol (100nM) incubation.

4.9 <u>Insulin effects on transcription factor gene expression.</u>

Since ABCA1 gene expression had been shown to be controlled by nuclear receptors and SCAN domain containing proteins. PPARs, KAP1, SDP1, ZNF202, ZNF195 and LXR-α mRNA expressions were determined after insulin incubation in a time kinetic schedule in human derived macrophages. PPARγ was upregulated early by insulin from the first hour (146% ± 17.3% P <0.05) till the fourth hour of incubation (153% ± 13.3% P <0.05) with a peak after the third hour of insulin incubation (217% ± 14.4% P <0.05) (Tab.10-A) and (Fig.13). Also with SDP1, insulin had upregulated SDP1 which was started from the first hour (156% ± 5.3% P <0.05) till the fourth hour (171% ± 5.6% P <0.05) with a maximum influence after the third hour (243% ± 17.4% P <0.05) (Tab.10-A) and (Fig.13). Insulin had also upregulated LXR-α which was started from the first hour (156% ± 3.5% P <0.05) till the fourth hour (180% ± 15.5% P <0.05) with a peak after the third hour of insulin incubation (214% ± 6.1% P <0.05) (Tab.10-B) and (Fig.13). Insulin had no influences on mRNA expression of PPARα, PPARδ, ZNF195, ZNF202 or KAP1.

	PPAR-α		**PPAR-γ**		**PPAR -δ**		**SDP1**	
	Value %	SD %	Value %	SD %	Value %	SD %	Value %	SD %
1h.	104.6*	6.7	145.7*	17.3	94.5*	6.9	155.5*	5.3
2h.	109.1*	5.8	185.8	12.3	102.5*	3.1	191.2*	11.5
3h.	98.5*	6.1	217.3*	14.4	103.3*	3.2	243.1*	17.4
4h.	105.8*	3.2	152.6*	13.3	101.4*	2.1	171.4*	5.6
6h.	95.9*	4.5	130.5*	17.2	99.5*	4.2	139.6	18.7
9h.	92.5*	5.8	112.4*	19.2	81.6*	1.9	102.5	19.9
18h.	96.4	6.2	104.3*	11.2	93.6	4	106.3*	6.6
24h.	91.4	5.5	95.3*	10.2	91.8	6.9	88.7	12.4

Table (10-A)

	KAP1		ZNF202		ZNF195		LXR-α	
	Value%	SD %	Value%	SD %	Value%	SD %	Value%	SD %
1h.	106.4*	3.4	104.4	3.9	105.5*	4.3	155.6*	3.5
2h.	95.7*	2.2	104.3*	1.9	99.4	12	193.8*	8.3
3h.	99.2	8.9	99.3*	2.9	88.3*	3.9	241.3*	6.1
4h.	115.1	7.9	91.4	3.8	100.3*	1.1	180.4*	15.5
6h.	95.6*	2.1	112.5*	2.7	95.4*	4.5	130.4*	6.1
9h.	100.2*	2.3	100.3	4.1	99.3	15	112.3*	9.1
18h.	82.5*	1.3	99.3*	3.8	120.5*	5.1	108.3	12.3
24h.	96.6*	3.5	85.3*	1.9	112.5*	2.2	99.3	9.1

Table (10-B)

Table(10):- PPARs, SDP1, KAP-1, ZNF202, ZNF195, and LXR-α genes expression in macrophages modulated by insulin.

Human macrophages were incubated in the presence of 150nM insulin for variable duration (1, 2, 3, 4, 6, 9, 18, and 24 h). PPARα, PPARβ, PPARγ, and SDP1 (Tab 10-A) and KAP-1, ZNF202, ZNF195, and LXR-α (Tab 10-B) mRNA expression levels were determined by TaqMan real time RT-PCR. Values are presented as mean oftriplicat valuein % compaired with macrophages and SD. * symbol means significant ($P \leq 0.05$).

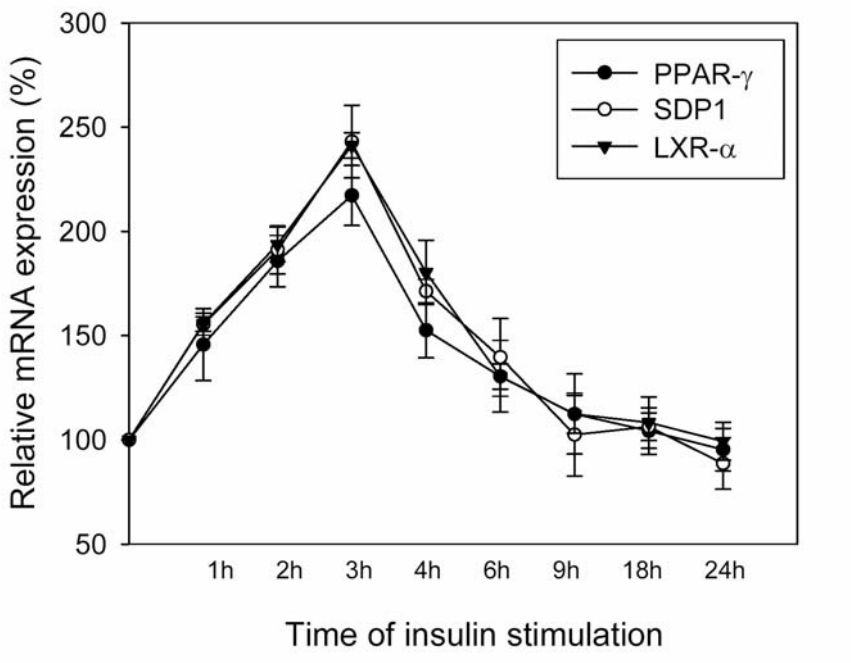

Fig. 13. PPARγ, SDP1, and LXR-α genes expression in macrophages modulated by insulin.

% of relative of PPARγ (•), SDP1 (o), and LXR-α (▼) mRNA expressions in human macrophages has been drawn in linear scale with vertical bars indicate SD in relation to variable duration of insulin (150 nM; for 1, 2, 3, 4, 6, 9, 18, and 24 h) incubation in macrophages.

4.10 ERs mRNA expression in macrophages and HepG2 cells

ERs mRNA in both HepG2 and human macrophages derived monocytes were relative quantificated by TaqMan real time PCR. ER-β was high expressed (ΔCT 9.5) in macrophages but ER-α was not expressed (ΔCT >20). In HepG2 cells, both ER-α and ER-β were low expressed (ΔCT 17.3 and 18.1 respectively) (Tab.11).

	ER-alpha		ER-Beta	
Type of cells	CT	ΔCT	CT	ΔCT
Macrophage	ne.	ne.	24.3	9.5
HepG2	31.7	17.3	32.9	18.1

Table (11):- ERs (ER-α and ER-β) gene expressions.
ERs gene expressions in both human monocytes derived macrophages and HepG2 cells were tested by TaqMan real time PCR. Values were presented as CT and Δ CT

5. Discussion

Hypercholesterolemia is one of the modifiable risk factors underlying ischemic heart disease, currently it is the most important cause of premature death in the world (Murray and Lopez, 1997). We have choosen human macrophages derived monocytes which are the source of foam cells responsible of pathogenesis of atherosclerosis and HepG2 cells because the liver cells are the major site for cholesterol biosynthesis and catabolism by converting cholesterol to bile acids (Shinji-Yokoyama, 2000) to examine the expression of their ABC transporters and their ApoA-1 dependent cholesterol and phospholipids effluxes.

In our results, ABC transporters expressions were tested in both human macrophages and HepG2 cells. ABCA4 is an active retinoid-PE-complex transporter, which displays strongly the lipid activated ATPase activity in photoreceptor cells (Ahn and Molday, 2000). ABCA4 was not expressed in both macrophages and HepG2 cells. ABCG5 and ABCG8 which implicat in the efflux of dietary sterols mainly plant sterols like sitosterol from intestinal epithelial cells back into the gut lumen and from the liver to the bile duct (Salen et al, 1970). Both ABCG5 and ABCG8 were not expressed in human macrophages but highly expressed in HepG2 cells.

ABCB1 can transport a variety of lipophilic cytotoxic drugs, cholesterol, phosphocholine, apoE and β-amyloid (Lavie et al, 2001 and Maggio-Price et al, 2002). It was higher expressed in HepG2 than in macrophage, and this could explain its protective action against excess accumulation of toxic substances by active translocation in both cell types. ABCB4 was highly expressed in HepG2 than macrophages because of its bile canalicular phosphatidylcholine translocase activity (De Vree et al, 1998). ABCB6, ABCB7, ABCB8, and ABCB10 are all targeted to the inner mitochondrial membrane and play a role in cellular iron homeostasis by transporting iron-sulfur (Fe/S) cluster precursor proteins (Zhang et al, 2000). Thez were mostly not expressed in HepG2 except ABCB10 and they were highly expressed in human macrophages except ABCB7. This may indicated that one or more of

these genes has a specific tissue location to prevent excess intracellular iron accumulation. ABCB7 was not expressed in either HepG2 or human macrophages, so, it had no role in preventing haemosiderosis in macrophages or liver tissue. ABCB11 which was the major bile salt transporter in mammalian liver (Strautnieks et al, 1998) was highly expressed in HepG2 but not in macrophages. ABCC1 (at the hepatic basolateral membrane domain), ABCC2 (at the hepatic apical membrane domain) and ABCC6 (at the hepatic lateral membrane domain) were highly expressed in HepG2 cell line and higher than macrophages especially ABCC2.

Abnormalities in lipid and lipoprotein metabolism (eg, increased LDL and decreased HDL levels) and atherosclerotic cardiovascular disease are commonly seen in post-menopausal women have been attributed to the increased coronary heart disease related mortality in these individuals (Sacks and Walsh, 1994). Low estrogen level is the primary metabolic alteration observed in postmenopausal women, so, endogenous concentrations of estrogen may have fundamental roles in lipoprotein mediated development of atherosclerotic coronary heart disease. Estrogen therapy significantly elevates plasma HDL levels and decreased LDL concentrations, suggesting a favourable influence on the plasma lipoprotein profile (Lobb, 1991). In postmenopausal women under estrogen therapy, they have a lower relative risk of coronary events than postmenopausal women who are not on estrogen therapy (Grady el al, 1992). The mechanism by which estrogen raises HDL levels is not clearly understood. Plasma turnover studies (kinetic) have indicated that estrogen increasing HDL level is solely due to the increase in the production rate of HDL-protein and apoA-I (Walsh el al, 1994). Contrary to these observations, it is shown that the treatment of premenopausal women with estradiol results in decreased hepatic lipase activity and suggests that estrogen may increase HDL level by decreasing the rate of HDL catabolism (Tikkanen el al, 1982). Srivastava found that estrogen antiatherogenic actions may occur via ABCA1-mediated pathway, and circulating HDL levels may influence expression of ABCA1 in mice (Srivastava 2002).

In our study, it was clearly evident that β-estradiol (100nM for 36h) has increased the specific apoA-I dependent cholesterol and choline-phospholipid effluxes, these actions were mediated through upregulation of ABCA1 mRNA which started after 1h up to 12 h with a maximum effect after 3h of β-estradiol incubation. In addition an upregulation of ABCG1 mRNA started after 1h and continuous for 24h with a maximum action after 3h could be seen. No influence of β-estradiol on ABCA7 mRNA expression in human macrophages derived monocytes was detected. This rapid genomic onset of action is not only with ABCA1 gene expression but also with vascular endothelial growth factor (VEGF) mRNA in the rodent uterus (Hyder et al, 2000), Fibulin-3 mRNA (FIBN3; Blackburn et al, 2003) and WISP-2 (mRNA and protein was overexpressed in preneoplastic and cancerous cells of human breast) (Banerjee et al, 2003). In HepG2 cells, β-estradiol slightly increased specific apoA-I dependent cholesterol efflux after 36h incubation and this presumbly mediated through upregulation of ABCG1 mRNA but not ABCA1 or ABCA7 mRNA. The later was downregulated by β-estradiol (100nM for 36h) which presumably decreased apoA-I dependent phospholipid efflux in HepG2 cells.

Kramer and Wray found that ER-β mRNA and ER-β protein are detected in primary monocyte-derived macrophages and the changes in estrogen concentration do not alter estrogen receptor levels suggesting a lack of autoregulation (Kramer and Wray, 2002). Western analysis with the ER-α antibody produces no signal in the macrophages (Vegeto et al, 2001). In mice, estrogen, despite lowering the levels of HDL, can upregulat the hepatic ABCA1 mRNA (1.5-2 folds), and in the absence of ER-alpha, ER-beta could compensate for ER-alpha in ER-alpha-/- hepatic mice (Srivastava, 2002). These results are co-ordinated with our results where ER-β mRNA was expressed in human macrophage derived monocytes, but not ER-α mRNA. In HepG2 cells, both ER-α and ER-β mRNA were very low expressed, and this can explain the less positive action of estradiol on HepG2 cells than macrophages. By these results, we could say that positive effects of β-estradiol on specific apoA-I dependent

cholesterol and choline-phospholipid effluxes and ABCA1 and ABCG1 genes expression were mediated through ER-β.

From our results, both β-estradiol and progesteron in macrophages had an upregulation action on ABCC9 which could regulate insulin release (Bryan and Aguilar- Bryan, 1999), so, both hormones might have a role in regulation of insulin release. More recently, Jankowski and co-workers found this relation between increased insulin extraction (C-peptide and insulin) and oral contraceptives (Jankowski et al, 2004). Also, hyperinsulinemia is caused by the direct stimulation of progestins on insulin secretion from the pancreas (Belaisch and Hommais-loufrani, 1988).

Estrogen and progesterone have been shown to have impact on cystic fibrosis transmembrane conductance regulator (CFTR) gene expression, tone of smooth muscle in the airways, immune response, exhaled nitric oxide and cytology in the tracheobronchial epithelium (Johannesson et al, 2000). Estrogen can inhibit chloride secretion in intact monolayers lung alveoli by its action on CFTR chloride channel activity (Ashvani et al, 2000). Progesterone inhibites cAMP-activated chloride-efflux from rabbit acinar cells (Sweezey et al, 1998). ABCC7 (CFTR), which is controlled by cAMP and thereby enables ATP binding and hydrolysis at the nucleotide binding domains, can in turn control opening and closing of the chloride channel (Sheppard and Welsh, 1999). Mutations in ABCC7 (CFTR) cause cystic fibrosis by affecting numerous secretion processes. In our results, estradiol and progesterone upregulated ABCC7 mRNA expression in both HepG2 and macrophages and this supported the effect of estrogen and progesterone on chloride channel and secretion through its influence on ABCC7 (CFTR) gene expression.

ABCD subfamily especially ABCD1 was induced by nuclear hormone receptors (Fourcade et al, 2001), estrogen had upregulated ABCD2 and ABCD3 in macrophages and ABCD1, ABCD2 and ABCD3 in HepG2 cells. This may explain the role of ERs (especially ER-α) in ABCD1 gene expression.

From our result, estrogen had an upregulated ABCB1 gene expression especially in HepG2 cells. Also, progesteron has the same action on ABCB1 especially on human macrophages. ABCB1 can cotransport apoE and β-amyloid and thereby may contribute to the etiology of Alzheimer´s disease (Maggio-Price et al, 2002). HRT by estrogen and progesteron has to be protective against alzheimer disease. Estrogen stimulates nerve growth factors and can improve cerebral blood flow in post-menopausal women (Birge, 1996). Estrogen and progesteron prevent accumulation of apolipoprotein E4 which is one of the causes of AD beside several other factors modifying the neuronal injury leading to alzheimer disease (Birge, 1996) through upregulation of ABCB1 gene expression.

Postmenopausal women with hypercholesterolemia under combined oral estrogen and progesteron therapy can result in a more cardioprotective lipoprotein-lipid profile than that achieved with either therapy used alone (Darling et al, 1999). Our results indicated that progesterone (1μM for 60h.) augmented specific apoA-I dependent cholesterol and choline-phospholipid effluxes, these actions were mediated through upregulating ABCA1 and ABCG1 mRNA but had not ABCA7 mRNA expression in human macrophages derived monocytes. In HepG2 cells, pregesterone could not influence specific apoA-I dependent cholesterol and phospholipids effluxes, ABCA1, ABCA7 or ABCG1 gene expression.

Little is known about the atherogenic potential of testosterone which has frequently been made responsible for the gender difference in the onset of coronary heart disease. In clinical studies, testosterone is found to exert both beneficial and adverse effects on cardiovascular risk factors and vascular function. The increased use of testosterone for treatment of male hypogonadism, as a hormone replacement therapy for aging men, and its use in male contraception make the issue important of whether exogenous testosterone is pro- or antiatherogenic (Von Eckardstein, 1998). The major argument for the putative atherogenicity of testosterone is its lowering the high density lipoprotein HDL-cholesterol (Alexandersen et al, 1996). Numerous clinical and epidemiological studies have demonstrated the inverse

association between HDL cholesterol and the risk of coronary heart disease events (Gordon and Rifkind, 1989). Men have considerably lower levels of HDL cholesterol than women. Moreover, application of exogenous testosterone leads to a dose-dependent decrease of HDL cholesterol, whereas either surgical or chemical castration causes a significant increase of HDL cholesterol in men (Whitsel et al, 2001). Testosterone led to a dose dependent upregulation of SR-BI mRNA and the protein levels and consequently increased HDL3-induced cholesterol efflux from macrophages (Langer et al, 2002). A supraphysiological dose of testosterone, it can increase the expression of hepatic lipase (HL) in HepG2 cells (Langer et al, 2002). Moreover, testosterone has no influence on the expression of apoA-I in HepG2 cells and ABCA1 in either HepG2 cells or macrophages and these suggest that testosterone, despite lowering HDL cholesterol, intensifies reverse cholesterol transport and thereby exerts an anti-atherogenic rather than a pro-atherogenic action (Langer et al, 2002). Others suggeste that androgen is an atherogenic hormone by increasing human foam cell formation through elevating expression of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) but without significant action on LDL or scavenger receptor expression (Martin et al, 2003).

From our data, androgen (1μM for 60h) was observed that it had no significant influence on ApoA-1 dependent cholesterol efflux, ABCA1 or ABCG1 mRNA expression in human macrophages. In HepG2 cells specific apoA-I dependent cholesterol was slightly decreased may be due to its downregulation of ABCG1 mRNA but it had no impact on ABCA1 mRNA expression. Androgen had an upregulation influence on ABCB11 which was the major bile salt transporter in the human liver cells; this may be added to the explanation of antiatherogenic action of androgen by excess cholesterol excretion in the bile (Strautnieks et al, 1998).

Thyroid hormones is observed to affect cholesterol metabolism depending on the facts that hypercholesterolemia is a useful marker for the diagnosis of hypothyroidism and an indicator

for progress in myxdematous patients under thyroxin therapy (Mason et al, 1930). Thyroid hormones, T4 and T3, enhance hepatic synthesis of cholesterol (Rossner and Rosenqvist, 1974), fractional clearance rates of VLDL and LDL particles (Rossner and Rosenqvist, 1974) and the hepatic excretion of cholesterol (Miettinen, 1968). In patients with hyperthyroidism, the concentrations of HDL cholesterol are generally lower than those in patients with hypothyroidism (Scottolini et al. 1980). *In vivo* study in rodents found that thyroid hormone increases the concentrations of apoA-I protein and the corresponding mRNA (Mooradian et al, 1996). The same result is observed in human HepG2 cells (Vandenbrouck et al, 1995). Our data revealed that thyroxin had a negative lowering influence on ApoA-1 dependent cholesterol and phospholipids effluxes in human macrophages and HepG2 cells, these influences could be added to explanation of hypercholesterolemic state of hypothyroidism. The ApoA-1 dependent cholesterol efflux by thyroxin on macrophages is lower than that of HepG2 cells, this was presumbly due to its downregulation of ABCA1 (only in macrophages) and ABCG1 (in macrophages and HepG2 cells) genes expressions, these results were identical to more recent results from Huuskonen and co-workers (2004) where they found that ABCA1 transcriptional activity is suppressed by T3 in primary human fibroblasts through competition between TR/RXR and LXR/RXR heterodimers to suppress or activate DR-4 element of the ABCA1 promoter (Huuskonen et al, 2004). The phospholipids efflux by thyroxin on HepG2 cells was lower than that of macrophages; this may be due to more prominent downregulation of thyroxin on ABCA7 gene expressions in HepG2 cells.

Insulin is well known as an anabolic hormone that stimulates the translocation of several transporters to the plasma membrane, resulting in reduction in blood glucose. It is also a potent growth factor and antiapoptotic factor that can regulate gene expression in various cells (Wu et al, 1995). In DM, the cells have low glucose and low ATP levels due to loss of insulin action on glycolysis enzymes. The cells can compensate the low ATP yield by excess lipolysis and lipid oxidation which lead to excess mitochondrial energy production and

mitochondrial exhaustment. This may result in cellular ATP shortage, a process that likely enhances the programmed cell death in macrophages. Mitochondrial exhaustment may also inhibit mitochondrial 27-OH sterol synthesis and its export from the mitochondrion, a critical pathway for LXR activation in response to cellular cholesterol stress (Fu et al, 2001). Since deficiency of 27-OH sterol which is the predominant oxysterol in macrophage-derived foam cells and atherosclerostic lesions (Brown and Jessup, 1999), may be engaged in the pathophysiological mechanism of atherosclerosis associated DM. Ratajczak and co-workers found a robust response of insulin on gene expression in a relatively short amount of time (Ratajczak et al, 2001). Also Sartipy and Loskutoff base their experiments on the early effects of insulin on gene transcription (Sartipy and Loskutoff, 2003). In present study, the rapid influence of insulin was clear on ABCA1 and ABCG1 but not ABCA7 gene expressions and supported by the longer impact of insulin on ABCA1 protein expression in comparison to its gene expression.

Also, in our study, glucagon had a reverse influence not only on both ABCA1 gene and protein expression but also on ABCA7 and ABCG1 genes expression which could explain the negative action of glucagon on Apo-A1 dependent cholesterol and phosphocholine effluxes in human macrophage cell.

In Hepatoblastoma cell line (HepG2), ApoA-1 dependent cholesterol and phosphocholine effluxes were increased after 18 hours of insulin incubation. These results run barrel with insulin upregulation of ABCA1 gene expression after 18h. Considering the time kinetic of insulin on ABCA1 gene expression, the results show that 2 peaks of ABCA1 after 3 and 18h. ABCA7 was downregulated after 18h of insulin incubation and this can explain the more positive augmented action of insulin on ApoA-1 dependent cholesterol efflux than on ApoA-1 dependent phosphocholine efflux.

Sartipy and Loskutoff found that insulin downregulated ABCA1 gene expression in human adipocytes after 3 hours insulin incubation by using Affymetrix GeneChips. In our

experiments, the data revealed that insulin had upregulation of ABCA1 gene expression in human macrophages after 3 hours insulin incubation and in HepG2 cells where ABCA1 gene expression has two peaks after 3 and 18 hours of insulin incubation by using TaqMan™ real time RT-PCR. These opposite results are due to a dose difference used in both experiments where Sartipy and Loskutoff used 1000 nM and we used 100 nM insulin concentrations. ABCA1 gene expression was significantly upregulated by variable insulin concentration (25 nM -250 nM), and reached its peak at insulin concentration level 150 nM then gradually decreased % of relative ABCA1 gene expression with increasing of insulin concentration above 150 nM. This indicates a variable insulin effect depending on both its concentration and may be on the type of cells (Sartipy and Loskutoff, 2003).

Patients with type-1 diabetes have increased cardiovascular risk even with normal or slightly elevated levels of HDL-cholesterol (Valabhji et al, 2002). Insulin reduces intracellular fatty acids by inhibiting intracellular hormone sensitive lipase enzyme especially in adipocyte with reduction of intracellular lipolysis of triglyceride (Anthonsen et al, 1998). Insulin deficiency in type 1 diabetes is associated with excess accumulation of fatty acids due to excess intracellular lipase. Excess unsaturated fatty acids downregulate the ABCA1 gene expression through inhibition of the nuclear transcription factor LXR-α (Costet et al, 2000). Insulin deficiency in type 1 diabetes is also associated with excess oxidation of fatty acids and excess yield of active acetate which is the source of ketone bodies formation (ketogenesis). Ketoacidosis not only suppresses ABCA1 gene expression (by acetoacetate) which is obvious in macrophage than in hepatocyte (Uehara, 2002) but also decreases hepatic ApoA-1 gene expression (by butyrate) in rat (Haas et al, 2000). Accumulation of butyrate has no action on ABCA1 gene expression (Uehara, 2002).

Insulin upregulated transcription and posttranscription of ABCA1. Transcription activation of ABCA1 mRNA by insulin was extended from 2-4 hours with high peak at 150 nM in Macrophages cells. Posttranscription activation of ABCA1 protein was extended for a longer

time up to 12 hours in Macrophages cells. This *in vitro* transcription can explained by antilipolyic activity of insulin with reduction of intracellular fatty acids especially unsaturated fatty acids which downregulate ABCA1 gene expression via suppression of the nuclear transcription factor LXR-α (Wang and Oram, 2002) or by its antiketogenic of insulin with less acetoacetate which can suppress ABCA1 gene expression (Uehara, 2002). Posttranscription effect of insulin on ABCA1 protein expression was related to its transcription action on ABCA1 mRNA expression but its prolongation for 12 hours presumably due to one of two reasons; first reason may be related to insulin stimulation of glycolysis with excess release of ATP which acts as a source of energy for ABCA1. However, this explanation can not be accepted because ABCA1 is a very low dependent ATP (Szakacs et al, 2001). Second reason may be related to reduction of intracellular unsaturated fatty acids which reduce the macrophage ABCA1 protein contents by enhancing its degradation rate (Wang and Oram, 2002).

Insulin induces a cascade of intracellular events in the adipocyte (Whiteman and Birnbaum, 2003) will start with binding to the α-subunits of its receptor at the cell surface and then activating the intrinsic tyrosine kinase activity of the β-subunits of the receptor. This interaction leads to phosphorylation of intracellular proteins, including insulin receptor substrate-1–4, GAB-1, and Cbl. The insulin signals subsequently diverge through different pathways that control distinct functions such as glucose transport, glucose/lipid metabolism, cell growth, protein synthesis, and gene expression (Feve et al, 1994). Although, the exact molecular mechanism that governs these pathways is not known, some key molecules have been identified. For example, numerous studies have confirmed a role for phosphatidyl inositol 3-kinase (Balbis et al, 2003) and mitogen-activated protein (MAP) protein kinase (O'Brien et al, 2000) in metabolic signalling. However, the actions of insulin on gene expression often occur independently of PI(3)K activity and may instead require activation of the Ras-Raf-MAPK pathway (Ezure et al, 1997). Since the activation of MAP kinase

signalling via insulin has been shown to be a rapid and transient event (O'Brien et al, 2000). ABCA1 gene induction by insulin occurs only in a narrow time window. In this study, we defined the exact pathway involved in ABCA1 upregulation by preincubation of human macrophage with a selective MAP kinase inhibitor for one hour had abolished ABCA1 upregulation action of insulin, but preincubation of human macrophage PI(3)K inhibitor had not abolish this action (Lida el al, 2001). This would prove that ABCA1 upregulation of insulin can proceed through MAP-Kinase dependent pathway. Insulin dependent MAP-Kinase pathway could explain the rapid and transient action of insulin on upregulation of ABCA1 gene expression (O'Brien et al, 2000).

Peroxisome proliferator activated receptors (PPARs) are nuclear receptors that regulate lipid and glucose metabolism and cellular differentiation. PPARγ plays an important role in adipocyte differentiation and regulation of adipocyte gene expression (Chinetti et al, 2001). PPARγ activators induce ABCA1 gene expression through LXR-α which is an oxysterol activated nuclear receptor inducing ABCA1- promotor transcription (Chinetti et al, 2001). Zhang and co-workers found that MAP kinase is an important mediator of cross-talk between insulin signal transduction pathways and PPARγ (Zhang et al, 2001). On the other hand Watanabe and co-workers observed that in adipocytes MAP-kinase activation, achieved by overexpression of MEK1, can inhibit PPARγ activity (Watanabe et al, 2003). So the relation between MAP-kinase and PPARγ is controversial. In our result, insulin upregulated PPARγ (but not PPARα or PPARδ) gene expression with a high peak after 3 hours. We had demonstrated that insulin stimulated transcriptional activity of PPARγ and ABCA1 mediated by MAP kinase in human macrophages. So, there might be a direct relation of MAP-kinase activating by insulin hormone and PPARγ gene expression.

Scan-domain containing protein (SDP1) shares in a high degree of amino acids sequence and previously identified as PPARγ2 co-activator in the mouse (Babb and Browen, 2003). Robert and Bowen in found that SDP1 bound through its SCAN-domain with non SCAN domain

containing protein PPARγ2 at its DNA-binding/ hinge region (Babb, and Bowen, 2003). So, SDP1 contributes to a PPARγ2 co-activator. Also SDP1 is shown to disrupt the binding of the transcription repressor KAP-1 to ZNF202 in vitro (Porsch-Ozcurumez et al, 2001). In our result, insulin has a stimulatory influence on SDP1 gene expression with a peak after 3 hours in human macrophage but not on ZNF202 or ZNF195 gene expression. SDP1 had an indirect upregulating ABCA1 gene expression through PPARγ2 activation and disrupting KAP-1 binding to ZNF202. ZNF202 mRNA expression is inversely correlated with ABCA1 (Porsch-Ozcurumez et al, 2001), ABCG1 and apoE (Langmann et al, 2003) in human monocytes.

The oxysterol receptor LXR (liver X receptor) is a nuclear receptor that plays a key role in regulation of cholesterol and fatty acid metabolism. LXR also plays a significant role not only in glucose metabolism (Guoqing et al, 2003) but also in lipid metabolism and cholesterol transport including ABCA1 (Costet et al, 2000), ABCG1 (Kennedy et al, 2001), ABCG5, ABCG8 (Repa et al, 2002), ApoE (Cao et al, 2002), CETP (Luo and Tall, 2000), Cyp7a (Edwards et al, 2002), LPL (Zhang et al, 2001), SREBP1c (Janowski et al, 1996), and FAS (Shao and Lazar, 1997). In our results, LXR-α mRNA was upregulated by insulin in a time dependent manner similar to that of ABCA1 gene upregulation by insulin. LXR-α induction by insulin might act on ABCA1 promotor site (DR4) to upregulat ABCA1 gene expression (Repa et al, 2002), and on ABCG1 promtor 3 to upregulat ABCG1 (Kennedy et al, 2001). The transcripitonal regulation of ABCD1 gene was also dependent on nuclear hormone receptor ligands, especially LXR ligands and PPAR ligands (Fourcade et al, 2001) and this could explane insulin upregulation effect on ABCD1 in HepG2 cells.

The mechanisms of glucocorticoids action on atherogenesis are poorly understood. Glucocorticoids seem to be anti-atherosclerotic in experimental animals by inhibiting leukocyte accumulation in the rabbit carotid artery and its intimal thickening (Hagihara et al, 1991), suppressing proliferation of macrophages and formation of foam cells in plaques (Asai et al, 1993) and markedly inhibiting cholesterol synthesis in various tissues presumably

through the inhibition of both HMG-CoA reductase and synthase activities (Lehoux et al,1989). Atherogenic action of glucocoticoids in human may be due to stimulation of the synthesis of apoB-100 and apoB-48, decrease their intracellular degradation (Wang et al,1995), increase serum cholesterol and its intracellular movement in human SMC (Brindley et al,1993), promote cholesterol esteification (Picard, et al.1981) and reduce HDL_3-mediated cholesterol efflux (Petrichenko et al, 1997). In our result, hydrocortisone decreased ApoA-1 cholesterol efflux in macrophages due to downregulation of ABCA-1. Also dexamethasone had more suppression impact on ApoA-1 cholesterol and phospholipids effluxes in HepG2 cells due to downregulation of ABCA-1 and more marked on ABCA7 and ABCG1 genes expressions. These influences might contribute to atherogenic effect glucocorticids especially with dexamethasone.

Corticosteroids are widely administered both antenatally and postnatally to stimulate lung maturation. Components of this response include acceleration of epithelial cell maturation to be more able to absorb lung liquid at birth (Ingbar, et al 1997) and secrete pulmonary surfactant (Ballard, et al 1997). In the neonatal rat, exogenous corticosteroids accelerate thinning of the alveolar wall and microvascular maturation, but inhibit the growth of new interalveolar septa (Massaro and Massaro, 1986). The inhibition of septal growth results in an emphysematous state of the lung in the mature animal, with a reduced number of enlarged alveoli (Tschanz and Burri, 1997). In the same species, glucocorticoid (dexamethasone) administration is found to inhibit surfactant-A and -C genes (Tschanz and Burri, 1997) and proteins (Fussel and Kelly, 1991) expression in the lung, which can explain the reduced formation of septa. Exogenous corticosteroids have a similar inhibitory action on alveolar formation in the fetal rhesus monkey. Inhibition of septal formation by corticosteroids is a result of inhibited elastin synthesis in the lungs (Noguchi, et al 1990). However, another study provided that corticosteroids have no or even upregulation effect on elastin synthesis (Anceschi, et al 1992). In our results, we found that corticosteroids (especially

dexamethasone) upregulated ABCA3 on both human macrophages and HepG2 cells. ABCA3 had a transporter action on surfactant phospholipids in alveoli.

Dexamethasone induces a decrease in insulin sensitivity and a proportionate increase in insulin secretion and in insulin concentrations in healthy individuals (Nicod et al, 2003). This can be correlated with our data with upregulation influence of dexamethasone on ABCC9 mRNA in macrophages.

ABCE1 participates in innate immune defence (Bisbal et al, 2001) and involves in the control of immune reaction. ABCF1 also shares this features with ABCE1 (Tzyack et al, 2000). Both ABCE1 and ABCF1 proteins may be a part of inflammatory processes related to rheumatoid arthritis (Richard et al, 1998). It is clearly evident that dexamethasone and hydrocortisone downregulated both genes in both HepG2 and most obvious in macrophages so, it can explain the benefit of use of glucocorticoids in treatment of rheumatoid arthritis (van den Brink et al, 1994).

The human luteinizing hormone (lutropin or LH) was normally inhibited by estrogen, specifically estradiol. However, when estrogen reaches a certain level, there is positive feedback to the anterior pituitary, resulting in an increase in circulating LH (Hill et al, 1980). Lewis and Wexler found that depression of circulating LH levels parallels the severity of the arteriosclerosis in rat (Lewis and Wexler, 1975). In our results, LH has a significant augmentation on ApoA-1 dependent phospholipids efflux in HepG2 cells which presumably related to its upregulation of ABCA7 gene expression but without influence on ABCA1 or ABCG1 gene expression.

Somatostatin (SRIF) is a peptide hormone and has a potent inhibiting growth hormone (GH) secretion, but less potent in inhibiting glucagon, insulin, gastrin, TSH, ACTH, secretin, pancreozymin, cholecystokinin, pepsin, and renin secretion (Brazeau et al, 1973). Changes in plasma lipids can be observed after treatment of acromegaly with somatostatin analogue (Octreotide) (Lancranjan et al, 1996). Serum cholesterol remaines unchanged but serum

triglyceride decreases and apoA-I increases after treatment (Oscarsson et al, 1994). A decrease in triglyceride (James et al, 1991), a rise in HDL cholesterol (Lam et al, 1993) and the decrease in the elevated apolipoprotein (a) levels (Lam, et al.1993) are also observed after octreotide treatment. But there are no reported data on the effect of lowering GH on small dense LDL and RLP in patients with acromegaly. In our data, somatostatin had a positive augmentation on ApoA-1 dependent phospholipids efflux in HepG2 cells, this action might correlate to its upregulation of ABCA7 and ABCG1 genes expressions (but without action on ABCA1 gene expression). This might be responsible for elevation of HDL cholesterol level (Lam, et al.1993) during its therapy in treatment of acromegaly.

octreotide administration in young adults with newly diagnosed diabetes mellitus type 1 positively influences both the onset and duration of remission (Vondra K et al 2004). This could be explained from our data that somatostatin had upregulated ABCC9 in macrophages. ABCC9 stimulates pancreatic β-cells insulin secretion (Bryan and Aguilar-Bryan, 1999).

6. Summary

Although the knowledge of ATP-binding cassette of lipid transport had grown substantially over the last few years, the detailed molecular mechanisms and the exact functions of these transporters were still awaiting clarification. In this thesis, analysis of the actions of human steroid and non-steroid hormones on cholesterol and phospholipid effluxes and on ABC transporters gene expression in two cell types (human monocytes derived macrophages and HepG2 cells) were performed. Human macrophages are the source of foam cells involved in the pathogenesis of atherosclerosis, and liver cells are the major site for cholesterol biosynthesis and catabolism by converting cholesterol to bile acids.

Some of these hormones had promoted ApoA-1 dependent cholesterol and phospholipid effluxes and others had suppressed these effluxes depending on their influences on the expression of ATP binding cassette transporters. β-Estradiol enhanced ApoA-1 dependent cholesterol and phospholipids effluxes in human macrophages through upregulation of ABCA1 and ABCG1 gene expression. In HepG2 cells, β-estradiol had little or reversed effect on ApoA-1 dependent cholesterol and phospholipids effluxes and sterol sensitive ABC transporters, which presumbly related to estrogen receptors gene expression. Estrogen receptor-β was high expressed in human macrophages but not in HepG2 cells. Upregulations of ABCA1 and ABCG1 genes expression by β-estadiol in human macrophages were time dependent where they started early and lasted for few hours.

Progesterone augmented ApoA-1 dependent cholesterol and phospholipids effluxes in human macrophages through upregulation of ABCA1 and ABCG1 gene expressions. Luteinizing hormone increased ApoA-1 dependent phospholipid efflux in HepG2 cells through augmentation of ABCA7 mRNA. Somatostatin hormone also enhanced ApoA-1 dependent phospholipid efflux in HepG2 cells by enhancing ABCA7 gene expression.

Human insulin promoted ApoA-1 dependent cholesterol and phospholipid effluxes in both human macrophages and HepG2 cells. These actions could be associated with an upregulation

of ABCA1 and ABCG1 mRNA. The ABCA1 upregulation of human insulin was time, concentration and tissue dependent. In human macrophages, ABCA1 and ABCG1 induction started early and extended over hours. The same occurred in HepG2 cells but with a more extended effect. The maximum concentration of insulin that could upregulate ABCA1 gene expression was 150 nM in human macrophages. Above this concentration, ABCA1 gene expression declined. Posttransscription influence of insulin was more pronounced than its influence on ABCA1 transcription. ABCA1 gene expression in human macrophages was induced by insulin via a MAP kinase signaling pathway. Furthermore, we speculate that activation of PPAR-γ and SDP1 were involved in this process.

Some hormones had suppressed ApoA-1 dependent cholesterol and phospholipid effluxes. Hydrocortisone suppressed cholesterol efflux in human macrophages presumbly by downregulation of ABCA1 gene expression. Triiodothyroxin decreased cholesterol efflux in both human macrophages and HepG2 cells through downregulation of ABCA1 and ABCG1 genes in human macrophages, and ABCA7 and ABCG1 genes in HepG2 cells. Glucagon declined ApoA-1 dependent cholesterol and phospholipid effluxes in human macrophages by supression of ABCA1, ABCA7 and ABCG1 gene expressions. Dexamethasone suppressed ApoA-1 dependent cholesterol and phospholipids effluxes in HepG2 cells through downregulation of ABCA1 and especially ABCA7 and ABCG1 genes expressions.

7. Reference List

Abele R., and Tampe R. (1999) Function of the transport complex TAP in cellular immune recognition. Biochim.Biophys.Acta 1461, 405-419.

Aguilar-Bryan L., Clement J., Gonzalez G., Kunjilwar K., Babenko A., and Bryan J. (1998) Toward understanding the assembly and structure of KATP channels. Physiol Rev. Jan; 78 (1):227-45.

Ahima R., Prabakaran D., Mantzoros C., Qu D., Lowell B., Maratos-Flier E., and Flier J. (1996) Role of leptin in the neuroendocrine response to fasting. Nature 382:250.

Ahn J., and Molday R. (2000) Purification and characterization of ABCR from bovine rod outer segments. Methods Enzymol. 315, 864-879.

Alexandersen P., Haarbo J., and Christiansen C. (1996) The relationship of natural androgens to coronary heart disease in males :a review, Atherosclerosis 125,1-3.

Allikmets R. (2000) Simple and complex ABCR: genetic predisposition to retinal disease. Am.J.Hum.Genet. 67, 793-799.

Allikmets R., Raskind W., Hutchinson A., Schueck N., Dean M., and Koeller D. (1999) Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anaemia and ataxia (XLSA/A). Hum.Mol.Genet. 8, 743-749.

Amherdt M., Patel Y., and Orci L., (1989) Binding and internalization of somatostatin, insulin, and glucagon by cultured rat islet cells. J Clin Invest 84:412–417.

Anber V., Griffin B., McConnell M., Packard C. and Shepherd J. (1996) Influence of plasma lipid and LDL-subfraction profile on the interaction between low density lipoprotein with human arterial wall proteoglycans. Atherosclerosis, 124, 261-271.

Anceschi M., Palmerini C., Codini M., Luzi P., and Cosmi E. (1992) Collagen and elastin in rabbit fetal lung: Ontogeny and effects of steroids. J. Dev. Physiol.18: 233–6.

Anne K., Daryl K., and Bruno C. (2000) Regulation by glucagon (cAMP) and insulin of the promoter of the human phosphoenolpyruvate carboxykinase gene (cytosolic) in cultured rat hepatocytes and in human hepatoblastoma cells. Biochem. J.352 ;211–217.

Anthonsen M., Ronnstrand L., Wernstedt C., Degerman E., Holm C. (1998) Identification of novel phosphorylation sites in hormone-sensitive lipase that are phosphorylated in response to isoproterenol and govern activation properties in vitro. J Biol Chem. Jan 2;273(1):215-21.

Asai K., Funaki C., Hayashi T., Yamada K., Naito M., Kuzuya M., Yoshida N., and Kuzuya F.(1993) Dexamethasone-induced suppression of aortic atherosclerosis in cholesterol-fed rabbits: possible mechanisms. Arterioscler Thromb.;13:892-899.

Ashvani K., Bruce D., John A., Elmer M., Robert J., and Neil A. (2000) Estrogen Inhibition of Cystic Fibrosis Transmembrane Conductance Regulator-Mediated Chloride Secretion. Vol. 295, Issue 1, 195-204, October.

Asztalos B., Brousseau M., McNamara J., Horvath K., Roheim P., and Schaefer E. (2001) Subpopulations of high density lipoproteins in homozygous and heterozygous Tangier disease. Atherosclerosis 156, 217-225.

Babb R., and Bowen B. (2003) SDP1 is a peroxisome-proliferator-activated receptor gamma 2 co-activator that binds through its SCAN domain. Biochem. J. 370:719-727.

Baelde H., Cleton-Jansen A., van Beerendonk H., Namba M., Bovee J., and Hogendoorn P. (2001) High quality RNA isolation from tumours with low cellularity and high extracellular matrix component for cDNA microarrays: application to chondrosarcoma. J. Clin. Pathol. 54:778-782.

Bakos E., Evers R., Szakacs G., Tusnady G., Welker E., Szabo K., de Haas M., van Deemter L., Borst P., Varadi A., and Sarkadi B. (1998) Functional multidrug resistance protein (MRP1) lacking the N-terminal transmembrane domain. J.Biol.Chem. 273, 32167-32175

Balbis A., Baquiran G., Dumas V., and Posner B. (2003) Effect of inhibiting vacuolar acidification on insulin signaling in hepatocytes. J Biol Chem. Dec 19.

Ballard P., Ning Y., Polk D., Ikegami M., and Jobe A. (1997) Glucocorticoid regulation of surfactant components in immature lambs. Am. J. Physiol. 273: L1048–57.

Banerjee S, Saxena N, Sengupta K, Tawfik O, Mayo MS, and Banerjee SK. (2003) WISP-2 gene in human breast cancer: estrogen and progesterone inducible expression and regulation of tumor cell proliferation. Neoplasia. Jan-Feb;5(1):63-73.

Belaisch J., and Hommais-loufrani B. (1988) Combined estrogen-progestagen contraception and glucid and water-sodium metabolism. Contracept Fertil Sex (Paris). Feb;16(2):3-7.

Bengtsson B., Eden S., Ernest I., Oden A., and Sjogren B. (1988) Epidemiology and long-term survival in acromegaly. Acta Medica Scandinavica, 223, 327-335.

Berry A., Scott H., Kudoh J., Talior I., Korostishevsky M., Wattenhofer M., Guipponi M., Barras C., Rossier C., Shibuya K., Wang J., Kawasaki K., Asakawa S., Minoshima S., Shimizu N., Antonarakis S., and Bonne-Tamir B. (2000) Refined localization of autosomal recessive nonsyndromic deafness DFNB10 locus using 34 novel microsatellite markers, genomic structure, and exclusion of six known genes in the region. Genomics 68, 22-29.

Bhattacharyya A. and Connor W. (1974) Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J.Clin.Invest 53, 1033-1043.

Birge SJ. (1996) Is there a role for estrogen replacement therapy in the prevention and treatment of dementia? J Am Geriatr Soc. Jul;44(7):865-70.

Bisbal C., Salehzada T., Silhol M., Martinand C., Le Roy F., and Lebleu B. (2001) The 2-5A/RNase L pathway and inhibition by RNase L inhibitor (RLI)Methods Mol.Biol. 160, 183-198.

Bjorkhem I., and Boberg K. (1999) Inborn errors in the bile acid biosythesis and storage of sterols other than cholesterol. The metabolic basis of inherited disease. 7, 2073-2102. Ref Type: Generic.

Blackburn J., Tarttelin EE., Gregory-Evans CY., Moosajee M., and Gregory-Evans K. (2003) Transcriptional regulation and expression of the dominant drusen gene FBLN3 (EFEMP1) in mammalian retina. Invest Ophthalmol Vis Sci. Nov;44(11):4613-21.

Bodyioch M., Orso E., Klucken J., Langmann T., Bottcher A., Diederich W., Drobnik W., Barlage S., Buchler C., Porsch O. M., Kaminski W. E., Hahmann H., Oette K., Rothe G., Aslanidis C., Lackner K. J., and Schmitz G. (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat.Genet. 22, 347-351.

Boffelli D., Zajchowski D., Yang Z., and Lawn R. (1999) Estrogen modulation of apolipoprotein(a) expression. Identification of a regulatory element. J Biol Chem. May 28;274(22):15569-74.

Bonne T.B., DeStefano A.L., Briggs C.E., Adair R., Franklyn B., Weiss S., Korostishevsky M., Frydman M., Baldwin C.T., and Farrer L.A. (1996) Linkage of congenital recessive deafness (gene DFNB10) to chromosome 21q22.3. Am.J.Hum.Genet. 58, 1254-1259.

Borst P., Evers R., Kool M., and Wijnholds J. (2000) A family of drug transporters: the multidrug resistance-associated proteins. J.Natl.Cancer Inst. 92, 1295-1302.

Bottcher A., Schlosser J., Kronenberg F., Dieplinger H., Knipping G., Lackner K., and Schmitz G. (2000) Preparative free-solution isotachophoresis for separation of human plasma lipo-proteins: apolipoprotein and lipid composition of HDL subfractions. J.Lipid Res. 41, 905-915.

Boulton T., Nye S., Robbins D., Ip N., Radziejewska E., Morgenbesser S., DePinho R., Panayotatos N., Cobb M., and Yancopoulos G. (1991). ERKs:a family of protein serine/threonine kinase that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 65.663-675.

Brazeau P., Vale W., Burgus R., Ling N., Butcher M., Rivier J., and Guillemin R. (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77–79.

Bridley D., McCann B., Niaura R., Stoney C., and Suarez E. (1993) Stress and lipoprotein metabolism: modulators and mechanisms. Metabolism.;42(suppl 1):3-15.

Brindley D., and Salter A. (1991) Hormonal regulation of the hepatic low density lipoprotein receptor and catabolism of low density lipoproteins. Prog Lipid Res.30:349-360.

Brinkmeier M., and Camper S. (1997) Localization of somatostatin receptor genes on mouse chromosomes 2, 11, 12, 15, and 17: correlation with growth QTLs. Genomics 43:9–14.

Broccardo C., Luciani M., and Chimini G. (1999) The ABCA subclass of mammalian transporters. Biochim.Biophys.Acta 1461, 395-404.

Broccardo C., Osorio J., Luciani M., Schriml L., Prades C., Shulenin S., Arnould I., Naudin L., Lafargue C., Rosier M., Jordan B., Mattei M., Dean M., Denefle P., and Chimini G. (2001) Comparative analysis of the promoter structure and genomic organization of the human and mouse ABCA7 gene encoding a novel ABCA transporter. Cytogenet.Cell Genet. 92, 264-270.

Brown A., and Jessup W. (1999) Oxysterols and atherosclerosis. Atherosclerosis. Jan; 142 (1) :1-28.

Bryan A., Laffitte, Lily C., Chao, Jing L., Robert W., Sarah H., Sean B., Antonio C., Damien C., David J., Jon L., Enrique S., and Peter T. (2003) Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci U S A. 2003 Apr 29;100(9):5419-24. Epub Apr 15.

Bryan J., and Aguilar-Bryan L. (1999) Membrane topology of the amino-terminal region of the sulfonylurea receptor. Biochim.Biophys.Acta 1461, 285-303.

Buechler C., Boettcher A., Bared SM., Probst MC., and Schmitz G. (2002) The carboxyterminus of the ATP-binding cassette transporter A1 interacts with a beta2-syntrophin/utrophin complex. Biochem Biophys Res Commun. May 3;293(2):759-65.

Bungert S., Molday L., and Molday R. (2001) Membrane topology of the ATP binding cassette transporter ABCR and its relationship to ABC1 and related ABCA transporters: identification of N-linked glycosylation sites. J. Biol Chem. Jun 29;276(26):23539-46.

Campos H., Blijlevens E., McNamara J., Ordovas J., Posner B., Wilson P., Castelli W., and Schaefer E. (1992a) LDL particle size distribution. Results from the Framingham Offspring Study. Arterioscler Thromb. Dec;12(12):1410-9.

Cao G., Beyer, T., Yang X., Schmidt, R., Zhang Y., Bensch W., Kauffman R., Gao H., Ryan T., Liang Y., Eacho P., and Jiang X. (2002). Enlargement of high density lipoprotein in mice via liver X receptor activation requires apolipoprotein E and is abolished by cholesteryl ester transfer protein expression. J. Biol. Chem. 277, 39561-39565.

Carr B., Ohashi M., Parker CR J., and Simpson E. (1981) The role of cyclic adenosine 3',5'-monophosphate in cholesterol metabolism and steroidogenesis by the human fetal adrenal gland. J Clin Endocrinol Metab. Jun;52(6):1124-8.

Cavelier L., Qiu Y., Bielicki JK., Afzal V., Cheng JF., and Rubin EM. (2001) Regulation and activity of the human ABCA1 gene in transgenic mice. J Biol Chem. 2001 May 25;276(21):18046-51. Epub 2001 Mar 13.

Chawla A., Boisvert W., Lee C., Laffitte B., Barak Y., Joseph S., Liao D., Nagy L., Edwards P., Curtiss L., Evans R., and Tontonoz P. (2001) PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 7: 161 –171.

Chinetti G., Lestavel S., Bocher V., Remaley A., Neve B., Torra I., Teissier E., Minnich A., Jaye M., Duverger N., Brewer H., Fruchart J., Clavey V., and Staels B. (2001) PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med. Jan;7(1):53-8.

Clee S., Zwinderman A., Engert J., Zwarts K., Molhuizen H., Roomp K., Jukema J., van Wijland M., van Dam M., Hudson T., Brooks-Wilson A., Genest J., Kastelein J., and Hayden M. (2001) Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation 103, 1198-1205.

Costet P., Luo Y., Wang N., and Tall A. (2000) Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. J. Biol. Chem. 275, 28240-28245.

Cowley S., and Parker M. (1999) A comparison of transcriptional activation by ER alpha and ER beta. J Steroid Biochem Mol Biol 69:165-175.

Croop, J., Tiller G., Fletcher J., Lux M., Raab E., Goldenson D., Son D., Arciniegas S., and Wu R. (1997) Isolation and characterization of a mammalian homolog of the Drosophila white gene. Gene 185, 77-85.

Darling GM., Johns JA., McCloud PI., and Davis SR. (1999) Concurrent use of simvastatin and estrogen-progestin therapy compared with each therapy alone for hypercholesterolemia in postmenopausal women. Climacteric. Sep; 2(3): 181-8.

Dean M., Yannick H.,Allikmet R., and Chimini G. (2001) ABC transporters: From genes to diseases. J Lipid Res.42 1007-1017.

Desprts J-P., Moorjani S., Lupien P., Tremblay A., Nadeau A., and Bouchard C. (1990) Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis. 10: 497-511.

De Vree J. M., Jacquemin E., Sturm E., Cresteil D., Bosma P. J., Aten J., Deleuze J. F., Desrochers M., Burdelski M., Bernard O., Oude Elferink R., and Hadchouel M. (1998) Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc.Natl.Acad.Sci.U.S.A 95, 282-287.

Drobnik W., Borsukova H., Bottcher A., Pfeiffer A., Liebisch G., Schutz G., Schindler H., and Schmitz. G. (2002) Apo AI/ABCA1-dependent and HDL3-mediated lipid efflux from compositionally distinct cholesterol-based microdomains. Traffic. 3:268-278.

Dutta A., Parikh B., Maben R., and Melnykovych G. (1989) Increased dolichol content in glucocorticoid-sensitive human T-cell leukemia line grown in the presence of dexamethasone. Biochem Biophys Res Commun. Jan 16;158(1):163-9.

Edwards P., Kast H., and Anisfeld A. (2002) BAREing it all: the adoption of LXR and FXR and their roles in lipid homeostasis. J. Lipid Res. 43, 2-12.

Engel, T., Lorkowski S., Lueken A., Rust S., Schluter B., Berger G., Cullen P., and Assmann G. (2001) The human ABCG4 gene is regulated by oxysterols and retinoids in monocyte-derived macrophages. Biochem.Biophys.Res.Commun. 288, 483-488.

Ezure T., Ishiwata T., Asano G., Tanaka S., and Yokomuro K.(1997) Production of macrophage colony-stimulating factor by murine liver in vivo. Cytokine. Jan;9(1):53-8.

Faredin I., and Toth I. (1975) The metabolism of [4-14C]5-androstene-3 beta, 17 beta-diol by normal human skin in vitro. Acta Med Acad Sci Hung. 32(2):139-52.

Fehmann H., Wulbrand U., and Arnold R. (2000) Treatment of endocrine gastroentero-pancreatic tumors with somatostatin analogues. Recent Results Cancer Res 153:15–22.

Feve B., Elhadri K., Quignard-Boulange A., and Pairault J. (1994) Transcriptional down-regulation by insulin of the beta 3-adrenergic receptor expression in 3T3-F442A adipocytes: a mechanism for repressing the cAMP signaling pathway. Proc Natl Acad Sci U S A. Jun 7;91(12):5677-81.

Forestier C., Frangne N., Eggmann T., and Klein M. (2003) Differential sensitivity of plant and yeast MRP (ABCC)-mediated organic anion transport processes towards sulfonylureas FEBS Lett. Nov 6;554(1-2):23-9.

Fourcade S., Savary S., Albet S., Gauthe D., Gondcaille C., Pineau T., Bellenger J., Bentejac M., Holzinger A., Berger J., and Bugaut M. (2001) Fibrate induction of the adrenoleukodystrophy-related gene (ABCD2): promoter analysis and role of the peroxisome proliferator-activated receptor PPARalpha. Eur.J.Biochem. 268, 3490-3500.

Friedman -Einat M., Boswell T., Horev G., Girishvarma G., Dunn I., Talbot R., and Sharp P. (1999) The chicken leptin gene: has it been cloned? Gen Comp Endocrinol. Sep;115(3):354-63.

Fussel JC., and Kelly FJ.(1991) Effects of dexamethasone on lung protein turnover. Biochem. J. 273: 93–7. 83.

Fu X., Menke JG., Chen Y., Zhou G., MacNaul KL., Wright SD., Sparrow CP., and Lund EG. (2001) 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. J Biol Chem. Oct 19;276(42):38378-87.

Gan X., Kaplan R., Menke JG., MacNaul K., Chen Y., Sparrow CP., Zhou G., Wright SD., and Cai T. (2001) Dual mechanisms of ABCA1 regulation by geranylgeranyl pyrophosphate.J Biol Chem 276:48702 –48708.

Godsland IF. (2001) Effects of postmenopausal hormone replacement therapy on lipid, lipo-protein, and apolipoprotein (a) concentrations: analysis of studies published from 1974-2000. Fertil Steril. May;75(5):898-915.

Golden PL., Maccagnan TJ., and Pardridge WM. (1997) Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. J Clin Invest. Jan 1;99(1):14-8.

Gordon, D. and Rifkind B. (1989) Current concepts:high density lipoproteins. The clinical implications of recent studies, N.Engl. J.Med.321 1311-315.

Grady D., Rubin SM., Petitti DB., Fox CS., Black D., Ettinger B., Ernster VL., and Cummings SR. (1992) Hormone therapy to prevent disease and prolong life in postmenopausal women. Ann Intern Med.117:1016 –1037.

Guoqing C., Yu L., Carol L., Brian A., Thomas P., Robert J., Youyan Z., Keith R., Chen S., Keith A., Anne R., Jiannong D., Patricia Foxworthy, Hong G., Timothy P., Xian-Cheng J., Thomas P., Patrick I., and Garret J. (2003) Antidiabetic Action of a Liver X Receptor Agonist Mediated By Inhibition of Hepatic Gluconeogenesis. J. Biol. Chem., Vol. 278, Issue 2, 1131-1136.

Haas M., Pun K., Reinacher D., Wong N., and Mooradian A. (2000) Effects of ketoacidosis on rat apolipoprotein A1 gene expression: a link with acidosis but not with ketones. J Mol Endocrinol. Aug;25(1):129-39.

Hagihara H., Nomoto A., Mutoh S., Yamaguchi I., and Ono T. (1991) Role of anti-inflammatory responses in experimental atherosclerosis: effects of anti-inflammatory drugs on cuff-induced leukocyte accumulation and intimal thickening of rabbit carotid artery. Atherosclerosis.91:107-116.

Hall E., Hylemon P., Vlahcevic Z., Mallonee D., Valerie K., Avadhani N., and Pandak W. (2001) Overexpression of CYP27 in hepatic and extrahepatic cells: role in the regulation of cholesterol homeostasis. Am J Physiol Gastrointest Liver Physiol. Jul;281(1):G293-301.

Hardardottir I., Grunfeld C.and FeingoldKR. (1994) Effects of endotoxin and cytokines on lipid metabolism. *Curr Opin Lipidol* **5** 3, pp. 207–215.

Heimberg M., Olubadewo JO., and Wilcox HG. (1985) Plasma lipoproteins and regulation of hepatic metabolism of fatty acids in altered thyroid states. Endocr Rev. Fall; 6(4): 590-607.

Herz J., and Beffert U. (2000) Apolipoprotein E receptors: linking brain development and Alzheimer's disease. Nat.Rev.Neurosci. 1, 51-58.

Higgins C., Gallagher M., Mimmack M., and Pearce S. (1988) A family of closely related ATP-binding subunits from prokaryotic and eukaryotic cells. Bioessays 8, 111-116

Hill P., Garbaczewski L., Helman P., Huskisson J., Sporangisa E., and Wynder E. (1980) Diet, lifestyle, and menstrual activity. Am J Clin Nutr. Jun;33(6):1192-8.

Hirohashi T., Suzuki H., and Sugiyama Y. (1999) Characterization of the transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3). J.Biol.Chem. 274, 15181-15185.

Hoggard N., Hunter L., Duncan J., Williams L., Trayhurn P., and Mercer JG (1997) Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. Proc Natl Acad Sci U S A. Sep 30;94(20):11073-8.

Huuskonen J., Vishnu M., Pullinger C., Fielding P., and Fielding C. (2004) Regulation of ATP-Binding Cassette Transporter A1 Transcription by Thyroid Hormone Receptor. Biochemistry. Feb 17;43(6):1626-32.

Hyde S., Emsley P., Hartshorn M., Mimmack M., Gileadi U., Pearce S., Gallagher M., Gill D., Hubbard R., and Higgins C. (1990) Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. Nature 346, 362-365.

Hyder SM., Huang JC., Nawaz Z., Boettger-Tong H., Makela S., Chiappetta C., and Stancel GM. (2000) Regulation of vascular endothelial growth factor expression by estrogens and progestins. Environ Health Perspect. Oct;108 Suppl 5:785-90.

Ingbar DH., Duvick S., and Savick SK.(1997) Developmental changes of fetal rat lung Na-K-ATPase after maternal treatment with dexamethasone. Am. J. Physiol. 272: L665–72.

Ito Y, Kozawa O., Tokuda H., Suzuki A., Watanabe Y., Kotoyori J., and Oiso Y. (1994) Glucocorticoid inhibits AMP production induced by vasoactive agents in aortic smooth muscle cells. Atherosclerosis.110:69-76.

Jafrati MD., Karas RH., Aronovitz M., Kim S., Sullivan Jr., Lubahn DB., O'Donnell Jr., Korach KS., and Mendelsohn ME. (1997) Estrogen inhibits the vascular injury response in estrogen receptor a-deficient mice. Nature Medicine 3 545- 548.

James R., Moller N., Chatterjee S., White M., and Kendall-Taylor P. (1991) Carbohydrate tolerance and serum lipids in acromegaly before and during treatment with high dose octreotide. Diabetic Medicine, 8, 517-523.

Jankowski CM., Ben-Ezra V., Gozansky WS., and Scheaffer SE. (2004) Effects of oral contraceptives on glucoregulatory responses to exercise. Metabolism. Mar;53(3):348-52.

Janowski B., Willy P., Devi T., Falck J., and Mangelsdorf D. (1996) An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature 383, 728-731.

Johannesson M., Ludviksdottir D., and Janson C. (2000) Lung function changes in relation to menstrual cycle in females with cystic fibrosis. Respir Med. Nov; 94(11): 1043-6.

Kamei Y., Xu L., and Heinzel T. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403-414.

Kaminski W., Orso E., Diederich W., Klucken J., Drobnik W., and Schmitz G. (2000) Identification of a novel human sterol-sensitive ATP-binding cassette transporter (ABCA7). Biochem.Biophys.Res.Commun. 273, 532-538.

Kaminski W., Wenzel J., Piehler A., Langmann T., and Schmitz G. (2001) ABCA6, a novel a subclass ABC transporter. Biochem.Biophys.Res.Commun. 285, 1295-1301.

Kanamori H., Krieg S., Mao C., Di Pippo VA., Wang S., Zajchowski DA., and Shapiro DJ. (2000) Proteinase inhibitor 9, an inhibitor of granzyme B-mediated apoptosis, is a primary estrogen-inducible gene in human liver cells. J Biol Chem. Feb 25;275(8):5867-73.

Kartenbeck J., Leuschner U., Mayer R., and Keppler D. (1996) Absence of the canalicular isoform of the MRP gene-encoded conjugate export pump from the hepatocytes in Dubin-Johnson syndrome. Hepatology 23, 1061-1066.

Kato S., Endoh H., and Masuhiro Y. (1995) Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. Science 270:1491-1496.

Kennedy M. A., Venkateswaran A., Tarr P., Xenarios I., Kudoh J., Shimizu N., and Edwards P. (2001) Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein. J. Biol. Chem. 276, 39438-39447.

Kent-Osborne C., Hong (Holly) Zhao, and Suzanne A.W. (2000) Selective Estrogen Receptor Modulators: Structure, Function, and Clinical Use. Journal of Clinical Oncology, Vol 18, No 17 September,: pp 3172-3186.

Keppler D., and Konig J. (2000) Hepatic secretion of conjugated drugs and endogenous substances. Semin Liver Dis.;20(3):265-72.

Khaw K-T. (1996) Dehydroepiandrosterone, dehydroepiandrosterone sulfate and cardiovascular disease. J Endocrinol.150:S149 –53.

Kieffer TJ., Heller RS., and Habener JF. (1996) Leptin receptors expressed on pancreatic beta-cells. Biochem. Biophys. Res. Commun.224:522.

Kielar D., Kaminski WE., Liebisch G., Piehler A., Wenzel JJ., Mohle C., Heimerl S., Langmann T., Friedrich SO., Bottcher A., Barlage S., Drobnik W., Schmitz G. (2003) Adenosine triphosphate binding cassette (ABC) transporters are expressed and regulated during terminal keratinocyte differentiation: a potential role for ABCA7 in epidermal lipid reorganization. J Invest Dermatol. Sep;121(3):465-74.

Klein, I., Sarkadi B., and Varadi A. (1999) An inventory of the human ABC proteins. Biochim. Biophys. Acta 1461, 237-262.

Klein S., Coppack SW., Mohamed-Ali V., Landt M. (1996) Adipose tissue leptin production and plasma leptin kinetics in humans. Diabetes. Jul;45(7):984-7.

Klucken J., Buchler C., Orso E., Kaminski W.E., Porsch-Ozcurumez M., Liebisch G., Kapinsky M., Diederich W., Drobnik W., Dean M., Allikmets R., and Schmitz G. (2000). ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci U S A. 97:817-822.

Konig J., Rost D., Cui Y., and Keppler D. (1999) Characterization of the human multidrug resistance protein isoform MRP3 localized to the basolateral hepatocyte membrane. Hepatology 29, 1156-1163.

Kool M., van der L. M., de Haas M., Baas F., and Borst P. (1999) Expression of human MRP6, a homologue of the multidrug resistance protein gene MRP1, in tissues and cancer cells.Cancer Res. 59, 175-182.

Kramer PR., and Wray S. (2002) 17-Beta-estradiol regulates expression of genes that function in macrophage activation and cholesterol homeostasis. J Steroid Biochem Mol Biol. Jul;81(3):203-16.

Kumar V., and Chambon P. (1988) The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. Cell 55:145-156.

Kumar V., Green S., and Stack G. (1987): Functional domains of the human estrogen receptor. Cell 51:941-951.

Laffel L. (1999) Ketones bodies : a review of physiology , pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev 15:412-426.

Laharrague P., Larrouy D., Fontanilles A., Truel N., Campfield A., Tenenbaum R., Galitzky J., Corberand J., Penicaud L., and Casteilla L. (1998) High expression of leptin by human bone marrow adipocytes in primary culture. FASEB J. Jun;12(9):747-52.

Lam K., Pang R., Janus E., Kung A., and Wang C. (1993) Serum apolipoprotein (a) correlates with growth hormone levels in Chinese patients with acromegaly. Atherosclerosis, 104, 183-188.

Lancranjan I., Bruns C., Grass P., Jaquet P., Jervell J., Kendall-Taylor P., Lamberts S.W., Marbach P., Orskov H., Pagani G., Sheppard M. and Simionescu L. (1996) Sandostatin LAR: a promising therapeutic tool in the management of acromegalic patients. Metabolism, 45, 67-71.

Langer C., Gansz B., Goepfert C., Engel T., Uehara Y., von Dehn G., Jansen H., Assmann G., and von Eckardstein A. (2002) Testosterone up-regulates scavenger receptor BI and stimulates cholesterol efflux from macrophages. Biochem Biophys Res Commun. Sep 6;296(5):1051-7.

Langmann T., Klucken J., Reil M., Liebisch G., Luciani M. F., Chimini G., Kaminski W. E., and Schmitz G. (1999) Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. Biochem.Biophys.Res.Commun. 257, 29-33.

Langmann T., Porsch-Ozcurumez M., Heimerl S., Probst M., Moehle C., Taher M., Borsukova H., Kielar D., Kaminski W. E., Dittrich-Wengenroth E., and Schmitz G. (2002) Identification of sterol-independent regulatory elements in the human ATP-binding cassette transporter A1 promoter: role of Sp1/3, E-box binding factors, and an oncostatin M-responsive element. J.Biol.Chem. 26;277(17):14443-50.

Langmann T., Schumacher C., Morham S., Honer C., Heimerl S., Moehle C., and Schmitz G. (2003) ZNF202 is inversely regulated with its target genes ABCA1 and apoE during macrophage differentiation and foam cell formation. J Lipid Res. 2003 May;44(5):968-77.

Thomas Langmann, Richard Mauerer, Alexandra Zahn, Christoph Moehle, Mario Probst, Wolfgang Stremme and Gerd Schmitz. (2003) Real-Time Reverse Transcription-PCR Expression Profiling of the Complete Human ATP-Binding Cassette Transporter Superfamily in Various Tissues. Clinical Chemistry 49:2 230–238.

Lavie Y., Fiucci G., and Liscovitch M. (2001) Upregulation of caveolin in multidrug resistant cancer cells: functional implications. Adv.Drug Deliv.Rev. 49, 317-323.

Lawn RM., Wade DP., Garvin MR., Wang X., Schwartz K., Porter JG., Seilhamer JJ., Vaughan AM., and Oram JF. (1999) The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J Clin Invest. Oct;104(8):R25-31.

Lazar D.F., Russell J., Matthew J., Cynthia C., Steven B., and Keishi Y. (1995). Mitogen activated protein kinase. Kinase inhibation does not block the stimulation of glucose utilization by insulin.J.Biol.Chem.270.20801-20807.

Lee GH., Proenca R., Montez JM., Carroll KM., Darvishzadeh JG., Lee JI., and Friedman JM. (1996) Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632.

Lee P., and Shulman, M.D. (2002) Effects of progestins in different hormone replacement therapy formulations on estrogen-induced lipid changes in postmenopausal women. Am J Cardiol ;89(suppl):47E-55E.

Lee M. H., Lu K., Hazard S., Yu H., Shulenin S., Hidaka H., Kojima H., Allikmets R., Sakuma N., Pegoraro R., Srivastava A. K., Salen G., Dean M., and Patel S. B. (2001) Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat.Genet. 27, 79-83.

Lee W. L., Tay A., Ong H. T., Goh L. M., Monaco A., and Szepetowski P. (1998) Association of infantile convulsions with paroxysmal dyskinesias (ICCA syndrome): confirmation of linkage to human chromosome Hum.Genet. 103, 608-612.

Lehoux JG., Lefebvre A., De Medicis E., Belisle S., and Bellabarba D. (1989) The enhancing effect of adrenocorticotropin on adrenal 3-hydroxy-3-methylglutaryl coenzyme A reductase messenger ribonucleic acid level is inhibited by aminoglutethimide but not by cycloheximide. Endocrinology. Jul;125(1):158-64.

Lemoisson E., Cren H., and Goussard J. (1994) Chromatographic separation of eight progesterone receptor isoforms in human breast tumors, and detection by radioligand and monoclonal antibodies. Association with hsp90 and hsp70 heat shock proteins. Ann Biol Clin (Paris).52(6):433-42.

Leroy P., Dessolin S., Villageois P., Moon BC., Friedman JM., Ailhaud G., and Dani C. (1996) Expression of ob gene in adipose cells. Regulation by insulin. J. Biol. Chem. 271:2365.

Le Saux O., Urban Z., Tschuch C., Csiszar K., Bacchelli B., Quaglino D., Pasquali-Ronchetti I., Pope F. M., Richards A., Terry S., Bercovitch L., de Paepe A., and Boyd C. (2000) Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. Nat.Genet. 25, 223-227.

Lewis B., and Wexler B. (1975) Changes in LH and prolactin in arteriosclerotic femal breeder rats. Atherosclerosis. May-Jun;21(3):301-14.

Iida K., Shimano H., Kawakami Y., Sone H., Toyoshima H., Suzuki S., Asano T., Okuda Y., and Yamada N. (2001)Insulin up-regulates tumor necrosis factor-alpha production in macrophages through an extracellular-regulated kinase-dependent pathway. J Biol Chem. 2001 Aug 31;276(35):32531-7.

Lida M., Murakami T., Ishida K., Mizuno A., Kuwajima M., and Shima K. (1996) Substitution at codon 269 (glutamine --> proline) of the leptin receptor (OB-R) cDNA is the only mutation found in the Zucker fatty (fa/fa) rat. Biochem Biophys Res Commun. Jul 16;224(2):597-604.

Lobb RA. (1991) Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. J Clin Endocrinol Metab.;73:925–930.

Lorkowski S., Rust S., Engel T., Jung E., Tegelkamp K., Galinski E. A., Assmann G., and Cullen P. (2001) Genomic sequence and structure of the human ABCG1 (ABC8) gene. Biochem.Biophys.Res.Commun. 280, 121-131.

Luo Y., and Tall A. (2000) Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. J. Clin. Invest. 105, 513-520.

Madon J., Hagenbuch B., Landmann L., Meier P. J., and Stieger B. (2000) Transport function and hepatocellular localization of mrp6 in rat liver. Mol.Pharmacol. 57, 634-641.

Maggio-Price L., Shows D., Waggie K., Burich A., Zeng W., Escobar S., Morrissey P., and Viney J. L. (2002) Helicobacter bilis infection accelerates and H. hepaticus infection delays the development of colitis in multiple drug resistance-deficient (mdr1a-/-) mice. Am.J.Pathol. Feb;160(2):739-51.

Magoffin DA., and Erickson GF. (1988) An improved method for primary culture of ovarian androgen-producing cells in serum-free medium: effect of lipoproteins, insulin, and insulinlike growth factor-I. In Vitro Cell Dev Biol. Sep;24(9):862-70.

Mandarino L., Stenner D., Blanchard W., Nissen S., Gerich J., Ling N., Brazeau P., Bohlen P., Esch F., and Guillemin R. (1981) Selective effects of somatostatin-14, -25 and -28 on in vitro insulin and glucagon secretion. Nature 291:76–77.

Mangelsdorf DJ., Thummel C., Beato M., Herrlich P., Schutz G., Umesono K., Blumberg B., Kastner P., Mark M., Chambon P., and Evans RE. (1995) The nuclear receptor superfamily: the second decade. Cell 83 835-839.

Martin K., Mbbs F., Shirley N., Anna B., Wendy J., David J., and David S. (2003) Dehydroepiandrosterone, an Adrenal Androgen, Increases Human Foam Cell Formation A Potentially ProAtherogenic Effect. J. of the American College of Cardiol. Vol. 42, No. 11, 1967-74.

Mason RL., Hunt HM., and Hurxthal L. (1930) Blood cholesterol values in hyperthyroidism and hypothyroidism – their significance. New England Journal of Medicine 203 1273–1277.

Massaro D., and Massaro GD. (1986) Dexamethasone accelerates postnatal alveolar wall thinning and alters wall composition. Am. J. Physiol.; 251: R218–24.

Masuoka H., Kamei S., Ozaki M., Kawasaki A., Shintani U., Ito M., and Nakano T. (2000) Predictive value of remnant-like particle cholesterol as an indicator of coronary artery stenosis in patients with normal serum triglyceride levels. Intern Med. Jul;39(7):540-6.

Matsui I., Nambu S., and Baba S. (1998) Evaluation of fasting serum insulin levels among Japanese school-age children. J Nutr Sci Vitaminol (Tokyo). Dec;44(6):819-28.

McDonnell D., Clemm D., and Hermann T. (1995) Analysis of estrogen receptor function in vitro reveals distinct classes of antiestrogens. Mol Endocrinol 9:659-668.

McEwen BS., Biron CA., Brunson KW., Bulloch K., Chambers WH. and Dhabhar FS. (1997) The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. *Brain Res Brain Res Rev* 23 1–2, pp. 79–133.

Miettinen TA. (1968) Mechanism of serum cholesterol reduction by thyroid hormones in hypothyroidism. Journal of Laboratory and Clinical Medicine 71 537–547.

Miyake K., Mickley L., Litman T., Zhan Z., Robey R., Cristensen B., Brangi M., Greenberger L., Dean M., Fojo T., and Bates S. (1999) An ATP-binding cassette gene (ABCG3) closely related to the multidrug transporter ABCG2 (MXR/ABCP) has an unusual ATP-binding domain. Cancer Res. 59, 8-13.

Mizuno TM., Bergen H., Funabashi T., Kleopoulos SP., Zhong YG., Bauman WA., and Mobbs CV. (1996) Obese gene expression: reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. Proc. Natl. Acad. Sci. USA 93:3434.

Montano M., Muller V., and Trobaugh A. (1995) The carboxy-terminal F domain of the human estrogen receptor: Role in the transcriptional activity of the receptor and the effectiveness of antiestrogens as estrogen antagonists. Mol Endocrinol 9:814-825.

Mooradian AD., Shuh GN., and Wong NCW. (1996) Age-related changes in the responsiveness of apolipoprotein AI to thyroid hormone. American Journal of Physiology 271 R1602–R1607.

Mosser J., Douar A., Sarde C., Kioschis P., Feil R., Moser H., Poustka A., Mandel J., and Aubourg P. (1993) Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. Nature 361, 726-730.

Muller M., and Jansen P. (1998) The secretory function of the liver: new aspects of hepatobiliary transport. J.Hepatol. 28, 344-354

Murray C., and Lopez AD. (1997) Mortality by cause for eight regions of the world: global burden of disease study. Lancet 349 1469–1504.

Murthy S., Born E., Mathur SN., and Field FJ. (2002) LXR/RXR activation enhances basolateral efflux of cholesterol in CaCo-2 cells. J Lipid Res. Jul;43(7):1054-64.

Naito M., Yasue M., Asai K., Yamada K., Hayashi T., Kuzuya M., Funaki C., Yoshimine N., and Kuzuya F. (1992) Effect of dexamethasone on experimental athero-sclerosis in cholesterol-fed rabbits. J Nutr Sci Vitaminol (Tokyo);38:255-264.

Nakamura MT., and Nara TY. (2002) Gene regulation of mammalian desaturases. Biochem Soc Trans. Nov; 30(Pt 6): 1076-9.

Nansel DD., Aiyer MS., Meinzer WH., and Bogdanove EM. (1979) Rapid direct effects of castration and androgen treatment on luteinizing hormone-releasing hormone-induced luteinizing hormone release in the phenobarbital-treated male rat: examination of the role direct and indirect androgen feedback mechanism might play in the physiological control of luteinizing hormone release. Endocrinology. Feb;104(2):524-31.

Neufeld EB., Demosky SJ., Stonik JA., Combs C., Remaley AT., Duverger N., Santamarina-Fojo S., and Brewer HB. (2002) The ABCA1 transporter functions on the basolateral surface of hepatocytes. Biochem Biophys Res Commun. Oct 4;297(4):974-9.

Neufeld EB., Remaley AT., Demosky SJ., Stonik JA., Cooney AM., Comly M., Dwyer NK., Zhang M., Blanchette-Mackie J., and Brewer HB. (2001) Cellular localization and trafficking of the human ABCA1 transporter. J Biol Chem. Jul 20;276(29):27584-90.

Ng M., Jessup W., and Celermajer D. (2001) Sex-related differences in the regulation of macrophage cholesterol metabolism. Curr Opin Lipidol. Oct;12(5):505-10.

Nicod N., Giusti V., Besse C., and Tappy L. (2003) Metabolic adaptations to dexamethasone-induced insulin resistance in healthy volunteers. Obes Res. 11(5):625-31.

Noguchi A., Firsching K., Kursar JD., and Reddy R. (1990) Developmental changes of tropoelastin synthesis by rat pulmonary fibroblasts and effects of dexamethasone. Pediatr. Res.; 28: 379–82.

O'Brien R.M., Streeper R.S., Ayala J.E., Stadelmaier B.T., and Hornbuckle L.A. (2000) Insulin-regulated gene expression. Biochem. Soc. Trans. 29:552-558.

Ogawa S., Inoue S., and Watanabe T. (1998) The complete primary structure of human estrogen receptor beta (hER-beta) and its heterodimerization with ER alpha in vivo and in vitro. Biochem Biophys Res Commun 243:122-126.

Ohama T., Hirano K., Zhang Z., Aoki R., Tsujii K., Nakagawa-Toyama Y., Tsukamoto K., Ikegami C., Matsuyama A., Ishigami M., Sakai N., Hiraoka H., Ueda K., Yamashita S., and Matsuzawa Y. (2002) Dominant expression of ATP-binding cassette transporter-1 on basolateral surface of Caco-2 cells stimulated by LXR/RXR ligands. Biochem Biophys Res Commun. Aug 23;296(3):625-30.

Oliver W., Shenk J., Snaith M., Russell C., Plunket K., Bodkin N., Lewis M., Winegar D., Sznaidman M., Lambert M., and Xu H. (2001) A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci U S A. Apr 24;98(9):5306-11. Epub Apr 17.

Oram JF., and Lawn RM. (2001) ABCA1. The gatekeeper for eliminating excess tissue cholesterol. J Lipid Res. Aug;42(8):1173-9.

Oram JF., Lawn RM., Garvin MR., and Wade DP. (2000) ABCA1 is the cyclic AMP-inducible apo-lipoprotein receptor that mediates cholesterol secretion from macrophages. J.Biol Chem 275:34508 –34511.

Orso E., Broccardo C., Kaminski W. E., Bottcher A., Liebisch G., Drobnik W., Gotz A., Chambenoit O., Diederich W., Langmann T., Spruss T., Luciani M. F., Rothe G., Lackner K. J., Chimini G., and Schmitz G. (2000) Transport of lipids from golgi to plasma membrane is defective in tangier disease patients and Abc1-deficient mice. Nat.Genet. 24, 192-196.

Oscarsson J., Wiklund O., Jakobsson K.E., Petrusont B., and Bengtsson B.A. (1994) Serum lipoproteins in acromegaly before and 6-15 months after transsphenoidal adenomectomy. Clinical Endocrinology, 41, 603-608.

Paine K., and Flower D. R. (2000) The lipocalin website. Biochim.Biophys.Acta 1482, 351-352.

Panousis CG., and Zuckerman SH. (2000) Interferon gamma induces down regulation of Tangier disease gene (ATP-binding cassette transporter 1)in macrophage-derived foam cells.Arterioscler Thromb Vasc Biol 20: 1565 –1571.

Panwala C. M., Jones J. C., and Viney J. L. (1998) A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J.Immunol. 161, 5733-5744.

Pastan I., and Gottesman M. (1991) Multidrug resistance. Annu.Rev.Med. 42, 277-286.

Patel YC., Greenwood M., Panetta R., Hukovic N., Grigorakis S., Robertson LA., and Srikant CB. (1996) Molecular biology of somatostatin receptor subtypes. Metabolism 45:31–38.

Patti, M.E. and Kahn C.R. (1998) The insulin receptor-a critical link in glucose homeostasis and insulin action. J.Basic Clin.Physiol.Pharmacol 9.89-109.

Perez A., Wagner AM., Carreras G., Gimenez G., Sanchez-Quesada JL., Rigla M., Gomez-Gerique JA., and Pou JM. (2000) Prevalence and phenotypic distribution of dyslipidemia in type 1 diabetes mellitus: effect of glycemic control. Arch Intern Med. Oct 9;160(18):2756-62.

Petrichenko IE., Daret D., Kolpakova GV., Shakhov YA., and Larrue J. (1997) Glucocorticoids stimulate cholesteryl ester formation in human smooth muscle cells. Arterioscler Thromb Vasc Biol. Jun;17(6):1143-51.

Picard F., Duval D. and Homo F. (1981) Effect of 25-hydroxycholesterol and dexamethasone on sterol synthesis in isolated mouse thymocytes. J Steroid Biochem. ;15: 461-466.

Porsch-Ozcurumez M., Langmann T., Heimerl S., Borsukowa H., Kaminiski WE., Drobnik W., Honer C., Schumacher C., and Schmitz G. (2001) The zinc finger protein 202 (ZNF202) is a transcriptional repressor of ATP binding cassette transporter A1 (ABCA1)and ABCG1 gene expression and a modulator of cellular lipid efflux.J Biol Chem 276:12427 –12433.

Pratt WB., and Toft DO. (1997) Steroid receptor interactions with heat shock proteins and immunophilin chaperons. Endocrine Reviews 18 306-360.

Praydayrol L., Jornvall H., Mutt V., and Ribet A. (1980) N-terminally extended somatostatin: the primary structure of somatostatin-28. FEBS Lett 109:55-58.

Raggers R. J., Vogels I., and van Meer G. (2001) Multidrug-resistance P-glycoprotein (MDR1) secretes platelet-activating factor. Biochem.J. 357, 859-865.

Ratajczak J., Majka M., Kijowski J., Baj M., Pan Z., Marquez L., Janowska-Wieczorek A., and Ratajczak M. (2001) Biological significance of MAPK, AKT and JAK-STAT protein activation by various erythropoietic factors in normal human early erythroid cells. Br J Haematol. Oct;115(1):195-204.

Reed D., Korytko A., Hipkin R., Wehrenberg W., Schonbrunn A., and Cuttler L. (1999) Pituitary somatostatin receptor (sst)1–5 expression during rat development: age-dependent expression of sst2. Endocrinology 140:4739–4744.

Repa J., Berge K., Pomajzl C., Richardson J., Hobbs H., and Mangelsdorf D. (2002) Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. J. Biol. Chem. 18, 18.

Rice W., Burhans M., and Wispe J. (1989) Effect of oxygen exposure on ATP content of rat bronchoalveolar lavage. Pediatr.Res. 25, 396-398.

Richard M., Drouin R., and Beaulieu A. (1998) ABC50, a novel human ATP-binding cassette protein found in tumor necrosis factor-alpha-stimulated synoviocytes. Genomics 53, 137-145.

Rocchi E., Khodjakov A., Volk E., Yang C., Litman T., Bates S., and Schneider E. (2000) The product of the ABC half-transporter gene ABCG2 (BCRP/MXR/ABCP) is expressed in the plasma membrane. Biochem.Biophys.Res.Commun. 271, 42-46.

Rohner-Jeanrenaud F., and Jeanrenaud B. (1996) Obesity, leptin, and the brain. N. Engl. J. Med. 334:324.

Rossner S., and Rosenqvist U. (1974) Serum lipoproteins and the intravenous fat tolerance test in hypothyroid patients before and during substitution therapy. Atherosclerosis 20 365–381.

Rottman JN., Widom RL., Nadal-Ginard B., Mahdavi V. & Karathanasis SK. (1991) A retinoic acid-responsive element in the apolipoprotein gene distinguishes between two different retinoic acid response pathways. Molecular and Cellular Biology 11 3814–3820.

Rujescu D., Giegling I., Dahmen N., Szegedi A., Anghelescu I., Gietl A., Schafer M., Muller-Siecheneder F., Bondy B., and Moller H. J. (2000) Association study of suicidal behavior and affective disorders with a genetic polymorphism in ABCG1, a positional candidate on chromosome 21q22.3. Neuropsychobiology 42 Suppl 1, 22-25.

Sacks FM., and Walsh BW. (1994) Sex hormones and lipoprotein metabolism. Curr Opin Lipidol.;5:236 –240.

Salen G., Ahrens E. H., Jr., and Grundy S. M. (1970) Metabolism of beta-sitosterol in man. J.Clin.Invest 49, 952-967

Salen G., Shefer S., Nguyen L., Ness G., Tint G., and Shore V. Sitosterolemia. (1992) J.Lipid Res. 33, 945-955.

Saltiel AR., and Kahn CR. (2001) Insulin signalling and the regulation of glucose and lipid metabolism. Nature. Dec 13;414(6865):799-806.

Santamarina-Fojo S., Remaley AT., Neufeld EB., and Brewer HB. (2001) Regulation and intra-cellular trafficking of the ABCA1 transporter. J Lipid Res. Sep;42(9):1339-45.

Sartipy P., and Loskutoff DJ. (2003) Expression profiling identifies genes that continue to respond to insulin in adipocytes made insulin-resistant by treatment with tumor necrosis factor-alpha. J Biol Chem. Dec 26;278(52):52298-306.

Schaefer E. J., Brousseau M. E., Diffenderfer M. R., Cohn J. S., Welty F. K., O'Connor J., Dolnikowski G. G., Wang J., Hegele R. A., and Jones P. J. (2001) Cholesterol and apolipoprotein B metabolism in Tangier disease. Atherosclerosis 159, 231-236.

Schmitz G., Kaminski W. E., and Orso E. (2000) ABC transporters in cellular lipid trafficking.Curr. Opin.Lipidol. 11, 493-501.

Schmitz G., Langmann T., and Heimerl S. (2001) Role of ABCG1 and other ABCG family members in lipid metabolism. J.Lipid Res. 42, 1513-1520.

Schuetz J., Connelly M., Sun D., Paibir S., Flynn P., Srinivas R., Kumar A., and Fridland A. (1999) MRP4: A previously unidentified factor in resistance to nucleoside-based antiviral drugs. Nat.Med. 5, 1048-1051.

Scottolini A., Bhagavan N., Oshiro T., and Abe S. (1980) Serum HDL cholesterol concentrations in hypo- and hyperthyroidsm. Clinical Chemistry 26 584–587.

Shao D., and Lazar M. (1997) Peroxisome proliferator activated receptor gamma, CCAAT/ enhancer -binding protein alpha, and cell cycle status regulate the commitment to adipocyte differentiation. J. Biol. Chem. 272, 21473-21478.

Shephered P.R., Nave B.T., and Siddle K. (1995) Insulin stimulation of glycogen synthesis and glycogen synthetase activity is blocked by wortmannin and rapamycin in 3T3-L1 adipocytes: evidence for the involvement of phosphoinsitol 3- kinase and p70 ribosomol protein-S-6 kinase. Biochem. J. 305:25-28.

Sheppard D., and Welsh, M. (1999) Structure and function of the CFTR chloride channel. Physiol Rev. 79, S23-S45.

Shinji-Yokoyama (2000) Release of cellular cholesterol: molecular mechanism for cholesterol homeostasis in cells and in the body. Biochimica et Biophysica Acta 1529 231-244.

Silverman J. and Schrenk D. (1997) Hepatic canalicular membrane 4: expression of the multidrug resistance genes in the liver. FASEB J. 11, 308-313.

Singaraja RR., Bocher V., James ER., Clee SM., Zhang L., Leavitt BR., Tan B., Brooks-Wilson A., Kwok A., Bissada N., Yang Y., Liu G., and Tafuri SR. (2001) Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and ApoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. J Biol Chem. Sep 7;276(36):33969-79. Epub 2001 Jun 21.

Slavin B., Ong J., and Kern P. (1994) Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. J Lipid Res. Sep;35(9):1535-41.

Smit J., Schinkel A., Weert B., and Meijer D. (1998) Hepatobiliary and intestinal clearance of amphiphilic cationic drugs in mice in which both mdr1a and mdr1b genes have been disrupted. Br.J.Pharmacol. 124, 416-424.

Srivastava N. (2002) ATP binding cassette transporter A1--key roles in cellular lipid transport and atherosclerosis. Mol Cell Biochem. Aug;237(1-2):155-64.

Stange EF., Preclik G., Schneider A., Seiffer E., and Ditschunneit H. (1981) Hormonal regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and alkaline phosphatase in cultured intestinal mucosa. Biochim Biophys Acta. Dec 4;678(2):202-6.

Stange EF., Schneider A., Schusdziarra V., and Ditschuneit H. (1984) Inhibitory effects of somato-statin on growth and differentiation in cultured intestinal mucosa. Horm Metab Res. Feb;16(2):74-8.

Stoney CM., and West S. (1997) Lipids, personality and stress: mechanisms and modulators. In: M Hillbrand and RT Sptiz, Editors, Lipids and human behavior, American Psychological Society, Washington (DC), pp. 47–66.

Strautnieks S., Bull L., Knisely A., Kocoshis S., Dahl N., Arnell H., Sokal E., Dahan K., Childs S., Ling V., Tanner M., Kagalwalla A., Nemeth A., Pawlowska J., Baker A., Mieli-Vergani G., Freimer N., Gardiner R., and Thompson R. (1998) A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat.Genet. 20, 233-238.

Sweezey N., Tchepichev S., Gagnon S., Fertuck K., and O'Brodovich H. (1998) Female gender hormones regulate mRNA levels and function of the rat lung epithelial Na channel. Am J Physiol. Feb; 274(2 Pt 1): C379-86.

Szabo K., Szakacs G., Hegeds T., and Sarkadi B. (1999) Nucleotide occlusion in the human cystic fibrosis transmembrane conductance regulator. Different patterns in the two nucleotide binding domains. J.Biol.Chem. 274, 12209-12212.

Szakacs G., Langmann T., Ozvegy C., Orso E., Schmitz G., Varadi A., and Sarkadi B. (2001) Characterization of the ATPase cycle of human ABCA1: implications for its function as a regulator rather than an active transporter. Biochem Biophys Res Commun. Nov 16;288(5):1258-64.

Takahashi Y., and Smith J. (1999) Cholesterol efflux to apolipoprotein AI involves endocytosis and resecretion in a calcium-dependent pathway. Proc Natl Acad Sci U S A. Sep 28;96(20):11358-63.

Tall A., and Wang N. (2000) Tangier disease as a test of the reverse cholesterol transport hypothesis. J.Clin.Invest 106, 1205-1207

Tammur J., Prades C., Arnould I., Rzhetsky A., Hutchinson A., Adachi M., Schuetz J., Swoboda K., Ptacek L., Rosier M., Dean M., and Allikmets R. (2001) Two new genes from the human ATP-binding cassette transporter superfamily, ABCC11 and ABCC12, tandemly duplicated on chromosome 16q12. Gene 273, 89-96.

Tan K., Ai V., Chow W., Chau M., Leong L., and Lam K. (1999a) Influence of low density lipoprotein (LDL) subfraction profile and LDL oxidation on endothelium-dependent and independent vasodilation in patients with type 2 diabetes. Journal of Clinical Endocrinology and Metabolism, 84, 3212-3216.

Tartaglia LA., Dembski M., Weng X., Deng N., Culpepper J., Devos R., Richards GJ., Campfield LA., Clark FT., and Deeds J. (1995) Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263.

Taylor AH., Wishart P., Lawless DE., Raymond J., and Wong NC. (1996) Identification of functional positive and negative thyroid hormone-responsive elements in the rat apolipoprotein AI promoter. Biochemistry. Jun 25;35(25):8281-8.

Tikkanen MJ., Nikkila EA., Kuusi T., and Sipinen S. (1982) High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norges-trel. J Clin Endocrinol Metab.;54:1113–1117.

Tilly-Kiesi M., Kahri J., Pyörchlch T., Puolakka J., Luotola H., and Lappi M. (1997) Responses of HDL subclasses, Lp(AI) and Lp(AI:AII) levels and lipolytic enzyme activities to continuous oral estrogen-progestin and transdermal estrogen with cyclic progestin regimens in postmenopausal women. Atherosclerosis 129, pp. 249–259.

Tomaszuk A., Simpson C., and Williams G. (1996) Neuropeptide Y, the hypothalamus and the regulation of energy homeostasis. Horm. Res. 46:53.

Tomono S., Kawazu S., Kato N., Ishii C., Ito Y., Shimizu M., Shimoyama M., Nakano T., and Nakajima K. (1994) Uptake of remnant like particles (RLP) in diabetic patients from mouse peritoneal macrophages. Journal of Atherosclosis and Thrombosis,1, 98-102.

Tribble D.L., Holl L.G., Wood P.D., and Krauss R.M. (1992) Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis, 93, 189-199.

Tschanz SA., and Burri PH.(1997) Postnatal lung development and its impairment by glucocorticoids. Pediatr. Pulmonol. Suppl.; 16: 247–9. 66.

Tuck C.H., Holleran S., and Berglund L. (1997) Hormonal regulation of lipoprotein(a) levels: effects of estrogen replacement therapy on lipoprotein(a) and acute phase reactants in postmenopausal women. Arterioscler Thromb Vasc Biol 17, pp. 1822–1829.

Twickler T., Dallinga-Thie G.M., Zelissen P.M., Koppeschaar H., and Erkelens D.W. (2001) The atherogenic plasma remnant-like particle cholesterol concentration is increased in the fasting and postprandial state in active acromegalic patients. Clinical Endocrinology, 55, 69-75.

Tzyack J. K., Wang X., Belsham G., and Proud C. (2000) ABC50 interacts with eukaryotic initiation factor 2 and associates with the ribosome in an ATP-dependent manner. J.Biol.Chem. 275, 34131-34139.

Tzukerman M., Esty A., and Santisomere D. (1994) Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramo-lecular regions. Molecular Endocrinology 8:21-30.

Ueda, K., Matsuo M., Tanabe K., Morita K., Kioka N., and Amachi T. (1999) Comparative aspects of the function and mechanism of SUR1 and MDR1 proteins. Biochim.Biophys.Acta 1461, 305-313.

Uehara Y. (2002) Polyunsaturated fatty acids and acetoacetate downregulated the expression of the expression of the ATP-binding cassette transporter A1 .Diabetes 51 2922-28 October.

Valabhji J., Donovan J., McColl A., Schachter M., Richmond W., and Elkeles R. (2002) Rates of cholesterol esterification and esterified cholesterol net mass transfer between high-density lipoproteins and apolipoprotein B-containing lipoproteins in Type 1 diabetes. Diabet Med. May; 19(5):424-8.

Valensi P., Gaudey F., Parries J., and Attali JR. (1993) Glucagon and noradrenaline reduce erythrocyte deformability. Metabolism. Sep;42(9):1169-72.

van den Brink H., van Wijk M., Geertzen R., and Bijlsma J. (1994) Influence of corticosteroid pulse therapy on the serum levels of soluble interleukin 2 receptor, interleukin 6 and interleukin 8 in patients with rheumatoid arthritis. J Rheumatol. Mar;21(3):430-4. J Rheumatol. Mar;21(3):430-4.

Vandenbrouck Y., Janvier B., Loriette C., Bereziat G., and Mangeney-Andreani M. (1995) Thyroid hormone modulates apolipoprotein-AI gene expression at the post-transcriptional level in Hep G2 cells. Eur J Biochem. Jul 1;231(1):126-32.

van Helvoort A., Smith A., Sprong H., Fritzsche I., Schinkel A., Borst P., and van Meer G. (1996) MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 87, 507-517.

Vegeto E., Bonincontro C., Pollio G., Sala A., Viappiani S., Nardi F., Brusadelli A., Viviani B., Ciana P., and Maggi A. (2001) Estrogen prevents the lipopolysaccharide-induced inflammatory response in microglia, J. Neurosci. 21 1809–1818.

Venkateswaran A., Repa J., Lobaccaro J., Bronson A., Mangelsdorf D., and Edwards, P. (2000) Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. J.Biol.Chem. 275, 14700-14707.

Von Eckardstein A. (1998) Androgens, cardiovascular risk factors, and athero-sclerosis, in: E. Nieschlag, H.M. Behre (Eds), Testosterone:Action, Deficiency, Substitution, seconded, Springer, Berlin, New York, ,pp.229-58.

Vulevic B., Chen Z., Boyd J., Davis W., Walsh E., Belinsky M., and Tew K. (2001) Cloning and characterization of human adenosine 5'-triphosphate-binding cassette, sub-family A, transporter 2 (ABCA2). Cancer Res. 61, 3339-3347

Walker J., Saraste M., Runswick M., and Gay N. (1982) The amino acid sequence of the beta-subunit of ATP synthase from bovine heart mitochondria. EMBO J. 1, 945-951

Walsh B., Schiff I., Rosner B., Greenberg L., Ravnikar V., and Sacks F. (1991) Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 325, pp. 1196–1204.

Walsh B., Li H., and Sacks F. (1994) Effects of postmenopausal hormone replacement with oral and transdermal estrogen on high density lipoprotein metabolism. J Lipid Res.35:2083–2093.

Wang C., McLeod R., Yao Z., and Bridley D. (1995) Effect of dexamethasone on the synthesis, degradation, and secretion of apolipoprotein B in cultured rat hepatocytes. Arterioscler Thromb Vasc Biol.15:1481-1491.

Wang Y., and Oram JF. (2002) Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1.J Biol Chem 277:5692 –5697.

Warner B., Worgul T., Drago J., Demers L., Dufau M., Max D., and Santen R. (1983) Effect of very high dose D-leucine6-gonadotropin-releasing hormone proethylamide on the hypothalamic-pituitary testicular axis in patients with prostatic cancer. J Clin Invest. Jun;71(6):1842-53.

Watanabe M., Kouichi I., Katagiri H., AWata T., Oka Y. and Katayama S. (2003) Regulation of PPAR gamma transcripitional activity in 3T3 adipocytes. Biochem. and Biophys.Res.Comm. 300.429-436.

Webster N., Green S., and Tasset D. (1989) The transcription activation function located in the hormone-binding domain of the human oestrogen receptor is not encoded by a single exon. EMBO J 8:1441-1446.

Whiteman E., and Birnbaum MJ. (2003) Assaying tyrosine phosphorylation of insulin receptor and insulin receptor substrates. Methods Mol Med.83:119-26.

Whitsel E., Boyko E., Matsumoto A., Anawalt B., and Siscovick D. (2001) Intramuscular testosterone esters and plasma lipids in hypogonadal men: a meta-analysis, Am.J.Med.111, 261 –269.

Wu H., Tai T., Chuang L., Chiu K., and Lin B. (1995) CA-repeated microsatellite poly morphism of the glucokinase gene and its association with non-insulin-dependent diabetes mellitus in Taiwanese. Diabetes Res Clin Pract. Oct;30(1):21-6.

Yamano G., Funahashi H., Kawanami O., Zhao L., Ban N., Uchida Y., Morohoshi T., Ogawa J., and Shioda S. (2001) ABCA3 is a lamellar body membrane protein in human lung alveolar type II cells. FEBS Lett. Nov 16;508(2):221-5.

Yang XP., Freeman LA., Knapper CL., Amar MJ., Remaley A., Brewer HB Jr., and Santamarina-Fojo S. (2002) The E-box motif in the proximal ABCA1 promoter mediates transcriptional repression of the ABCA1 gene. J Lipid Res. Feb;43(2):297-306.

Zhang F., Zhang W., Liu L., Fisher C., Hui D., Childs S., Dorovini-Zis K., and Ling V. (2000) Characterization of ABCB9, an ATP binding cassette protein associated with lysosomes. J.Biol.Chem. 275, 23287-23294.

Zhang Y., Repa J., Gauthier K., and Mangelsdorf J. (2001) Regulation of Lipoprotein Lipase by the Oxysterol Receptors, LXR-α and LXR-β. J. Biol. Chem. 276, 43018-43024.

Zhou C., Zhao L., Inagaki N., Guan J., Nakajo S., Hirabayashi T., Kikuyama S., and Shioda S. (2001) ATP-binding cassette transporter ABC2/ABCA2 in the rat brain: a novel mammalian lysosome-associated membrane protein and a specific marker for oligodendrocytes but not for myelin sheaths. J.Neurosci. 21, 849-857.

Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe des Literaturzitats gekennzeichnet.

Weitere Personen waren an der inhaltlich-materiellen Herstellung der vorliegenden Arbeiten nicht beteiligt. Insbesondere habe ich hierfür nicht die entgeltliche Hilfe eines Promotionsberaters oder anderer Personen in Anspruch genommen. Niemand hat von mir wieder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

Regensburg, den 17.05.2004

(Mohamed A. Taher)